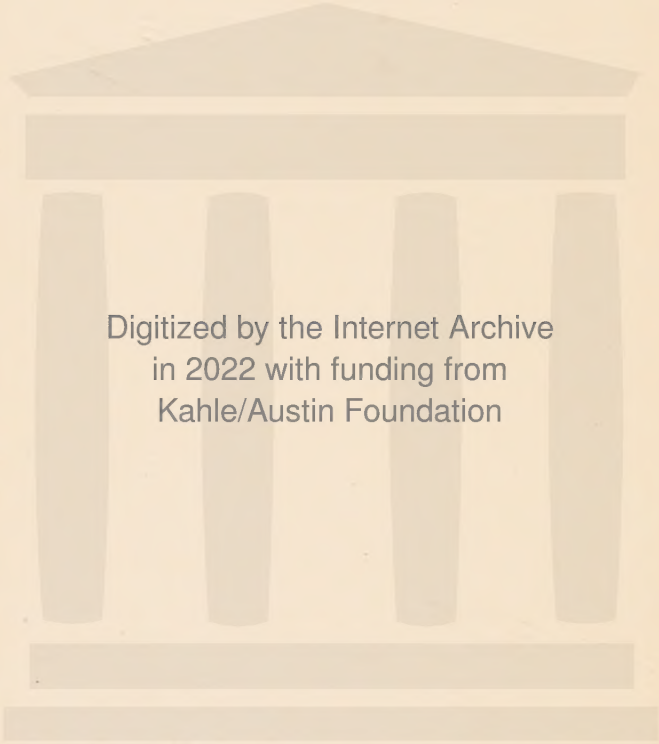

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RECENT ADVANCES IN ANALYTICAL CHEMISTRY

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RECENT ADVANCES IN ANALYTICAL CHEMISTRY

VOL. I—ORGANIC CHEMISTRY

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PREFACE

In these days workers require the latest information on matters connected with Analytical Chemistry arranged in an easily accessible form. To supply this want two volumes have been included in the Recent Advances Series—one on Organic Chemistry, and the other on Inorganic Chemistry. The present volume on Organic Chemistry is divided into sectional chapters which have been written by contributors with special knowledge of their subjects.

The general scheme of the work is to give a brief critical summary of the analytical methods (*i.e.*, mainly general principles) in use up to about eight or ten years ago, with references to original literature and English abstracts. From that period onwards, recent developments are dealt with more fully, and concise working details of new methods are given. Some suggestions are also made as to the directions in which future advances in analytical chemistry in the respective subjects are to be expected.

The work is primarily intended for those who have a general knowledge of chemistry but require information as to the best methods of dealing with analytical problems on the various fields discussed. It is thus not intended to replace, but to supplement, such works as " Allen's Organic Analysis."

C. AINSWORTH MITCHELL.

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RECENT ADVANCES IN ANALYTICAL CHEMISTRY

VOLUME I

CHAPTER I

SUGAR ANALYSIS

By C. L. Hinton, F.I.C.

Physical Methods: The Polarimeter The Saccharimeter Clarification Density Refraction Electrical Conductivity Colour Measurement. *Chemical Methods:* Gravimetric Copper Methods Volumetric Methods Other Reduction Methods Colorimetric Methods Biochemical Methods Mixed Sugar Analyses Certain Accessory Determinations Sugar Factory Methods Future Development of Sugar Analysis.

THE currently used methods of sugar analysis are very much matters of habit: have become, as it were, hereditary. The same, it is true, may be said of other branches of analytical practice, but, because of the relatively long history behind the main methods of sugar analysis, there has been time for them to become fixed and acquire the blessing of tradition. In face of the accumulated records of years, new methods or modifications must have very special attraction in order to displace the older and less exact.

The tendency has, no doubt, been helped by the existence of a large sugar-producing industry very much dependent on routine analytical operations, not to be varied capriciously, though known to be faulty. Moreover, this same industry is split up into several large areas of activity insularised by language and situation; Central Europe, the Dutch East Indies, the United States and Cuba, for instance. And, arising out of this segregation, there has been a distinct tendency for methods which may have originally been very similar, to develop along diverging lines in the different sugar areas. This can be seen in the Continental preference

for iodometric estimation of unreduced copper; the persistence of the French sugar scale for saccharimeters; and the adherence to gravimetric methods in America. The influence of the sugar industry has, of course, spread outside so as to affect workers interested in sugar analysis more generally; and lately, the special demands of biochemistry have led to a great further multiplication of methods. The labours of the International Commission for Uniform Methods of Sugar Analysis have been partially successful in arresting the divergence as far as the sugar trade is concerned; but its efforts have been put very much behind by the war, which, moreover, imposed an increased isolation upon the large continental area, and opened wider the gap between the lines of advance.

Essential principles are simple, and until the last decade or so have been confined mainly to the application of the polariscope and the use of copper solutions. But these have branched off in matters of detail until all sorts of minor and often arbitrary modifications have become endowed with ritualistic sanctity. As far as the polariscope is concerned, the industry is perhaps chiefly responsible. Its special requirements, combined with the importance of sucrose as a commercial article, have evolved that essentially arbitrary instrument the saccharimeter; and even about its arbitrariness there has been no unanimity, so that we have two or three "sugar scales" and "normal weights" and occasional proposals for still others.

Copper reduction methods have multiplied the more in detail through depending on reactions not having a stoichiometric basis. The work of numerous investigators, of whom the latest is Amick (*Brit. Chem. Abs.*, 1927, *A*, 1213), has thrown light on the extreme degree of dependence of these methods on conditions such as alkalinity. Unfortunately nearly everyone who examines the behaviour of alkaline copper solutions with sugars ends by proposing a new method for their use, and we have diversity continually being more diversified.

Thus the analyst, in general, fails to gain a benefit anything like commensurate with the vast amount of work devoted to developments and refinements in these main fields. The present survey may perhaps serve to stem the divergence somewhat by bringing

out the points of recent advance that can most usefully be added to or displace existing methods, and by placing some of the newer independent methods in correct relation to the old.

PHYSICAL METHODS

The Polarimeter. The polarimeter itself, like the analytical balance, is capable of a precision beyond that of most practical requirements. It is not to be wondered at, therefore, that changes in this instrument have been rare of late. An advance in one useful respect is presented by a polarimeter by Hilger which permits of readings with turbid liquids which cannot be read in the ordinary instrument.

It is more in regard to methods of illumination that there are improvements to record. A number of useful ideas for providing good sodium light have been proposed; one of the simplest and most practical is that of McLachlan and Middleton (*Analyst*, 1927, **52**, 639). Sticks of the following composition are used: Sodium chloride, 33; sodium bromide, 33; magnesium oxide, 14; and sodium silicate, 20 per cent. The first three are mixed to a thick paste with water; this is kneaded into the silicate, and the mixture is rolled out into sticks which are dried in the oven. The stick, ignited in a Bunsen flame, gives a brilliant sodium light. Schoorl (*Brit. Chem. Abs.*, 1926, *A*, 142, 264) has tried to secure a strong light by using a mixed solution of copper sulphate and potassium dichromate as a light-filter in conjunction with a metal-filament lamp with matt surface. This, he found, could replace a sodium light, with good results (to about 0·1°) for rotations of 10 to 15°.

As a source of monochromatic light the mercury vapour lamp has come into extensive use in the last few years. The line selected from the mercury spectrum (by means of an optical dispersion system) is the yellow-green line, $\lambda = 546$. This gives a very convenient light source, as to the wave-length of which there can be no suspicion.

In reading the polarimeter, most users have hitherto been content to use the eye, tiring as this may be for prolonged readings. A number of attempts have been made, with increasing success, to apply photo-electric devices to this end. Miss Dickes (*Int.*

Sugar J., 1927, 544) has described an apparatus capable of an accuracy of 0·01 for the red end of the spectrum. With the arrival of the new photo-cell (now on the market as a standard article) and the aid of modern methods of amplification, a reliable photo-electric polarimeter should soon be possible; giving the advantage of elimination of personal error, and perhaps a simplification of the optical parts. Kenyon (*Nature*, 1926, **117**, 304) even goes so far as to hint at the possibility of a recording polarimeter on these lines. The time is ripe for a general development of electrical methods for reading instruments depending on light transmission, such as colorimeters, etc. The scientific world already lags behind the commercial in the application of the photo-electric cell.

Fundamental Data.—The extended use of the mercury lamp has introduced a fresh crop of constants and specific rotation data. The specific rotations of a number of sugars for the mercury line, $\lambda = 546$, have now been determined with precision. For sucrose, Bates and Jackson's value at 20° C., for a concentration of 26 grms. per 100 c.c., is 78·34 (*Bur. of Standards Sci. Paper*, No. 268, 1916); whilst Jackson has determined that for dextrose as

$$(\alpha)^{20}_{\lambda = 546} = 62{\cdot}032 + 0{\cdot}0422\,p + 0{\cdot}0001897\,p^2,$$

(p = per cent. by weight *in vacuo*)

(*Bur. of Standards Bulletin*, No. 25; *Analyst*, 1927, **52**, 661).

Bacharach (*Analyst*, 1923, **48**, 521) found for lactose, with its temperature variation:

$$(\alpha)^{t}_{\lambda = 546} = 61{\cdot}94 + (20 - t) \times 0{\cdot}085.$$

Values for other sugars are still required, though they can be calculated fairly closely, by means of appropriate factors, from the rotations for the sodium D line. Vosburgh (*J. Amer. Chem. Soc.*, 1920, **42**, 1696; *Analyst*, 1920, **45**, 383) has determined factors for lævulose solutions, varying somewhat with concentration and temperature.

The specific rotation of lævulose for the D line (λ 589), about which there was disagreement, has also been reliably established

by Vosburgh (*loc. cit.*) and expressed as an equation combining concentration and temperature effects :

$$(\alpha)^{t}_{D} = -102{\cdot}65 - c\,(0{\cdot}220 - 0{\cdot}0028\,t) + 0{\cdot}00086c^2 + 0{\cdot}566\,t.$$

(c = grms. of lævulose per 100 c.c.)

This is valid for temperatures between 15° and 37° C., and for concentrations between 2·6 and 20 grms. per 100 c.c.

Using this equation in conjunction with Tollens' older data for dextrose, Zerban (*J. Amer. Chem. Soc.*, 1925, **47**, 1104 ; *Analyst*, 1925, **50**, 294) has calculated equations for the $(\alpha)_D$ of invert sugar solutions :

$$(\alpha)^{20}_{D} = -(19{\cdot}415 + 0{\cdot}07065\,c - 0{\cdot}00054\,c^2)$$

$$(\alpha)^{t}_{D} = (\alpha)^{20}_{D} + (0{\cdot}283 + 0{\cdot}0014\,c)\,(t - 20),$$

and has found a conversion factor for invert sugar, between the saccharimeter scale and the angular rotation for sodium light, of 0·34615.

These data enable the polarisation of mixtures involving invert sugar to be dealt with more confidently than before. In this connection, attention may be called to a useful principle in dealing with optical rotations in mixed solutions. The optical effect of each sugar, as affected by concentration, is that corresponding to the total sugar concentration of the solution. This was established experimentally by Vosburgh (*J. Amer. Chem. Soc.*, 1921, **43**, 219 ; *J. Soc. Chem. Ind.*, 1921, 233A), and its importance emphasised in a controversy between Jackson and Gillis, C. A. Browne, and Hinton (*Int. Sugar J.*, 1921, 22). Zerban made use of the principle in calculating his equation for invert sugar.

The Saccharimeter. Improvements in the quartz wedge saccharimeter recently have been of minor importance. Bellingham and Stanley (*Int. Sugar J.*, 1922, 587) have described an instrument in which troubles due to cementing of the prisms, fracture of the sensitive edge of the Lippich half-prism, and defects of the quartz wedges, have been surmounted by novel methods of construction.

Testimony is borne to the degree of precision of which the

saccharimeter is capable, by the amount of attention paid over a long period to the exact testing of the 100° point of the Ventzke scale. This point is the reading given in a 200-mm. tube at 20° C. by a solution of 26·000 grms. of pure sucrose (the "International normal weight") weighed in air with brass weights, in 100 c.c. of solution at 20° C. In practice, saccharimeter scales are calibrated against a "normal" quartz plate, which is one giving the same reading as the "normal" sucrose solution.

The latest report on this subject by Browne (*J. Assoc. Off. Agric. Chem.*, 1929, **12**, 106; *Chem. Abstr.*, 1929, **23**, 2058), describes the exhaustive tests carried out by the Bureau of Chemistry and the New York Sugar Trade Laboratory, proving that the 100° point of the Herzfeld-Schönrock scale, and therefore the readings of normal quartz plates based upon it, are too high by 0·10°; whilst Stanĕk (*Z. Zuckerind. Czechoslov.*, 1921, **45**, 417; *J. Soc. Chem. Ind.*, 1921, 711A) and Kraisy and Traegel (*Z. Ver. deut. Zuckerind.*, 1924, **74**, 193; *J. Soc. Chem. Ind.*, 1924, *B* 608) have found an even greater error. In the analysis of lower-grade products an error of this magnitude is probably more than balanced by other errors, but for high-purity products Browne and Zerban advocate changing the normal weight so as to avoid an alteration of the saccharimeter scale. As against this, it is contended by Dekker that the German scale should be retained on the grounds of uniformity, even if actually slightly in error (*Arch. Suikerind.*, 1928, **36**, II, 753.; *Chem. Abst.*, 1928, 4855). It seems desirable for the International Commission for Uniform Methods to decide upon this question.

Analytical Methods. The primary use of the polarimeter and saccharimeter in sugar analysis still remains the determination of sucrose. For this purpose, the direct reading in the saccharimeter of more or less pure products, to give directly the percentage of sucrose (a custom long and firmly established in the sugar industry, and for which the saccharimeter was, in fact, originally designed), has shown signs of giving place to more exact methods. Its limitations have become more and more evident: on the other hand, extended research has confirmed the value of the Clerget principle of polarisation before and after hydrolysis, which affords

a means of determination ranking as one of the most accurate of analytical methods.

For the attainment of this accuracy with impure sucrose products, the hydrolysis must be effected with invertase, which has no influence on the optical activity of any other constituents of the solution except raffinose (if present). The method is free from many objections to which the methods of acid hydrolysis are open; but until recently it had not found much favour, owing to the difficulty of obtaining reliable and uniform preparations of the enzyme, and to the relative slowness of the hydrolysis. These difficulties have now been met as a result of work by Reynolds (*J. Ind. Eng. Chem.*, 1924, **16**, 169; *Analyst*, 1924, **49**, 188) in perfecting a preparation capable of a hydrolysis as rapid as the ordinary hydrochloric acid inversion. The process has reached the commercial stage in America, and several standardised invertase preparations are on the market (Paine and Balch, *J. Ind. Eng. Chem.*, 1925, **17**, 240; *J. Soc. Chem. Ind.*, 1925, *B* 417). The invertase solution can be prepared in the laboratory from baker's yeast; a full description of its preparation, testing and use has been included in *Official Methods of Analysis*.

The value of the Clerget constant (for invertase) has been exactly determined by Paine and Balch (*J. Amer. Chem. Soc.*, 1927, **49**, 1019; *Analyst*, 1928, **53**, 350), who found the variation with concentration of sugar solution to be greater than hitherto accepted. The equation Constant = 131·17 − 0·073 *c* represents their results in terms of the saccharimeter scale. (This gives values, at sucrose concentrations of half normal and above, which are consistent with Zerban's calculated equation for the $(\alpha)_D^{20}$ of invert sugar. At lower concentrations there is a slight discrepancy.) In mixed solutions the concentration of the total sugar should be used in the formula, according to the principle already mentioned.

The only substance likely to interfere with the determination of sucrose is raffinose, which is present in small quantities in beet products, and which is hydrolysed by invertase to lævulose and melibiose. It is also hydrolysed to lævulose, dextrose, and

galactose by the enzyme melibiase, which occurs with invertase in bottom yeast, and the different behaviour with these enzymes has been made the basis of a means of estimating both sucrose and raffinose in beet products by Paine and Balch (*Ind. Eng. Chem.*, 1925, **17,** 240; *Int. Sugar J.*, 1925, 276). More recently the same workers have re-determined the constant for the hydrolysis of raffinose by invertase (*J. Amer. Chem. Soc.*, 1927, **49,** 1019; *Analyst*, 1928, **53,** 350).

The Clerget process was originally carried out with hydrochloric acid as the hydrolysing agent, and underwent modifications by various workers until, in 1888, it was closely standardised by Herzfeld in regard to concentrations of sugar and acid, and conditions of heating. He established a basic figure for the divisor and its variation with concentration of sugar. The Herzfeld process became the standard method for the determination of sucrose in sugar products, and it was long before certain defects in the method became so insistent as to compel a revision.

The whole of the procedure has been examined and put on a new basis of accuracy by Jackson and Gillis (*Bureau of Standards Sci. Paper*, No. 375, 1920; *Int. Sugar J.*, 1920, **22,** 509, 570, 638). They investigated the conditions of hydrolysis with hydrochloric acid, especially the Herzfeld procedure, which they found to require very careful handling if errors due to destruction of lævulose were to be avoided. The best temperature of inversion for rapid work was not above 60° C., and the following method was devised for inversion at this temperature:—

Fifty c.c. of the sugar solution are pipetted into a 100-c.c. flask, and diluted to 70 c.c. (or 70 c.c. of solution may be used). Ten c.c. of hydrochloric acid of strength 6·34*N* are added, the flask is immersed in a water bath at 60°, agitated continuously for about three minutes, and allowed to remain in the bath for a total time of nine minutes; it is then cooled quickly, and the contents made to volume at 20° for polarisation. (The strength of acid in the final solution is identical with that of the Herzfeld procedure.)

The basic value of the Clerget divisor, for like concentrations of sugar and acid to those of Herzfeld, was found by exhaustive experiments to be 143·25, and not 142·66, as found by him.

Further, Jackson and Gillis determined the exact influence of varying concentrations of hydrochloric acid and of certain salts on the optical rotations of sucrose and invert sugar. Their data are summarised in the following equations, for the positive and negative constituents of the divisor (referred to the half-normal weight of sucrose or inverted sucrose in 100 c.c.) :

Positive		*Negative*	
R	$= 100{\cdot}00$	R	$= -32{\cdot}00$
R_{NaCl}	$= 100 - 0{\cdot}265\ m$	R_{HCl}	$= -32{\cdot}00 - 0{\cdot}541\ m$
R_{NH_4Cl}	$= 100 - 0{\cdot}169\ m$	R_{NaCl}	$= -32{\cdot}00 - 0{\cdot}540\ m$
R_{CaCl_2}	$= 100 - 0{\cdot}339\ m$	R_{NH_4Cl}	$= -32{\cdot}00 - 0{\cdot}563\ m$
$R_{K_2C_2O_4}$	$= 100 - 0{\cdot}234\ m$		

(m is the amount in grms. of the salt or acid in 100 c.c. of solution as polarised.)

With this knowledge, they were able to apply with exactitude a principle due to Saillard (1913*a*). This consists in the neutralisation of the acid, after inversion, by sodium or ammonium hydroxide; at the same time, to the solution for direct polarisation is added an amount of salt equal to that formed by the neutralisation of the acid in the inverted solution. This eliminates errors due to optically active substances whose rotation is affected by the acidity of the solution. When such substances are absent, but invert sugar present in the uninverted solution, a modified procedure is adopted: the inverted solution is left unneutralised, but to the uninverted solution is added an amount of sodium chloride equal, in its effect on the rotation of the invert sugar, to the acid of the former. In this way there is no optical change due to the invert sugar initially present, but solely to the hydrolysis of the sucrose.

Tables of divisors, corrected for concentration and temperature effects, were computed, but these appear to have been based upon an erroneous method of allowing for concentration effects in mixed sugar solutions (*Int. Sugar J.*, 1921, 689; 1922, 313, 420) and are, therefore, of doubtful value. This is not of much importance, however, since the appropriate divisor can be readily calculated for any particular proportions of acid and salts, etc.,

from the essential data above given and the usual corrections for temperature and concentration. It is advisable always to make both polarisations at the same concentration of total sugars.

The utility of the method is, of course, not limited to determinations with the saccharimeter, for by conversion to corresponding equations for the effect on specific rotations of the sugars, the equations for the divisor can be applied to polarisations in angular degrees.

The applicability of these methods to solutions of the pure sugars only has been verified by several workers; and Monier-Williams has applied the neutral polarisation process successfully to mixtures of milk and sucrose (*Analyst*, 1928, **53**, 569). It is in regard to low-grade products such as molasses that difficulties still occur. Sázavský reported that the non-sugars of molasses lowered the effective concentration of acid and so caused inversion to be incomplete (*Listy Cukrovár.*, 1921, **39**, 409; *Chem. Abstr.*, 1922, 849). Later, he suggested the addition of sufficient extra acid to overcome the effects of the inorganic constituents of molasses (*Z. Zuckerind. Czechoslov.*, 1924, **48**, 247, 255; *J. Soc. Chem. Ind.*, 1924, *B*, 608). At the same time, the Walker method of inversion (*J. Soc. Chem Ind.*, 1917, 153) should be used, to avoid the formation of reversion products. To allow for the fact that mutarotation effects lag somewhat behind the actual hydrolysis, a period of about an hour should elapse before a reading is made.

The effect of non-sugars in modifying the conditions of inversion as well as the value of the divisor has been recognised by the Milk Products Sub-Committee of the Society of Public Analysts in their report on the determination of sucrose in condensed milk (*Analyst*, 1930, **55**, 111). In their standard process they have slightly adapted the Jackson and Gillis method of inversion, and have evaluated the inversion divisor applicable to the special case of condensed milk clarified according to defined procedure (see section on "*Milk Products*," p. 260).

Zerban has compared the Jackson and Gillis methods with the ordinary acid process and with invertase, for sugar syrups and molasses (*J. Assoc. Off. Agric. Chem.*, 1928, **11**, 167; 1929, **12**, 158; *Int. Sugar J.*, 1928, 443). If reversion products are already

present in a sample, then partial hydrolysis of these may occur, giving apparently high results for sucrose, even with inversion at room temperature. With inversion at 60°, slight action on any invert sugar initially present may cause a lowering of the sucrose figure. Thus a high or a low result is possible, or the two effects may at times balance, giving apparently correct results. The "neutral" process does not overcome these difficulties. His conclusion is that the only safe method for low-grade products is the invertase hydrolysis.

There have been ingenious attempts in another way to secure that the two polarisations shall be made in similar solutions. The idea was due to Deerr (*Int. Sugar J.*, 1915, **17**, 179), and has been modified by Coates and Shen (*Ind. Eng. Chem.*, 1928, **20**, 70; *Brit. Chem. Abstr.*, 1928, *B*, 423). The solution for direct polarisation is clarified by adding baryta followed by its equivalent of aluminium sulphate. This liberates aluminium hydroxide in the solution; at the same time all the baryta is removed. No interfering salts remain. Inversion is accomplished by means of sulphuric acid, and to the cooled solution the same amount of aluminium sulphate is added as in the direct polarisation, and the mixture is then exactly neutralised with baryta, thereby again clarifying and removing all reagents. Certain corrections for the volume of the precipitates have to be applied.

The method has decided attraction, but it still fails to overcome the troubles due to reversion products and invert sugar already present. It might, perhaps, be improved by applying the Walker method of inversion, with a readjustment of the quantity of acid and other reagents.

The alteration of the basic Clerget constant from Herzfeld's figure necessitated a revision of the latter's formulæ for calculating sucrose and raffinose in their mixtures. This was made by Browne and Gamble (*J. Ind. Eng. Chem.*, 1921, **13**, 793; *Analyst*, 1921, **46**, 456). Raffinose calculations have also been examined by Saillard (*Compt. rend.*, 1924, **178**, 2189; *Analyst*, 1924, **49**, 437).

There is a shadow of irony over the Clerget process in the circumstances of the moment. It seems possible that the acid inversion method is on the verge of obsolescence. It can hardly

be doubted that it must in time give place to the increasing simplicity and reliability of the newer use of invertase, after most of its difficulties have become known and a vast amount of painstaking work has eliminated many of them.

Few other specific methods for estimating sugars by the polarimeter, applicable in general circumstances, have been developed. Usually a combination of polarimetric and chemical methods has to be applied. In the case of lævulose, however, there is the possibility of estimation by taking advantage of the large temperature variation in its optical activity. The principle has been known and used for some time, but it has now been applied with success by Collins to solutions containing less than 1 per cent. of the sugar (*J. Soc. Chem. Ind.*, 1922, **41**, 561). The same principle has also been used by Jackson, Silsbee, and Profitt in their work on the preparation of lævulose (*Bur. of Standards Sci. Papers*, 1926, **20**, 587; *Analyst*, 1926, **51**, 304).

Clarification, etc., of Solutions. The traditional method for preparing impure sugar products for polarimetric analysis has been by treatment with a basic lead salt, such as the acetate or nitrate; but the possible errors introduced have always been a source of difficulty. Besides the uncertainty as to the volume occupied by the lead precipitate, there is the fact that the excess of defecant in the solution affects the polarisation of the sugars, and also that sugars may be removed from the solution by the lead, whether by occlusion or in the form of lead compounds. The latter is especially liable to occur if the basicity of the lead is too high, and when the alkali is added separately, as in Herles' basic nitrate method, this is difficult to control. Dorfmüller has shown the best way of effecting this, by adding the two reagents in portions at a time, so that there is at no time an excess of alkali localised within the mixture (*Z. Ver. deut. Zuckerind.*, 1924, 135; *Int. Sugar J.*, 1924, 278). Sommer has also recently studied this question in relation to molasses (*Z. Zuckerind. Czechoslov.*, 1928, **53**, 45; *Brit. Chem. Abstr.*, 1928, *B*, 939).

In spite of the disadvantages, the use of lead salts still maintains its hold. Minor modifications are from time to time suggested, some of these lately having consisted in reinforcing the action of

the lead with other reagents, *e.g.*, aluminium sulphate (Kalshoven and Sulmans, *Int. Sugar J.*, 1921, 627); and tannin (Sázavský, *Z. Zuckerind. Czechoslov.*, 1921, **45**, 227; *J. Chem. Soc.*, 1921, **120**, ii., 418).

For solutions which require less drastic decolorisation, Balch has found that hydrated aluminium silicate gives better results than alumina cream (*Sugar*, 1926, **28**, 551; *Int. Sugar J.*, 1927, 441). A novel method proposed by Schlemmer, and meriting further trial, consists in liberating bromine in the sugar solution by means of potassium bromide and chloramine-T (*Z. Zuckerind. Czechoslov.*, 1928, **53**, 13; *Brit. Chem. Abstr.*, 1928, *B*, 831).

In the special case of milk products, it has been found that clarification with zinc acetate and potassium ferrocyanide gives most satisfaction (see section on "*Milk Products*," p. 260).

For the present, however, the ideal method of clarifying, it must be confessed, is scarcely within sight.

Correcting for the volume of lead precipitate and other insoluble matter is often a harassing matter, especially in determinations of sugar in beet, etc. There has been some discussion in several quarters as to the correct allowance in such analysis, but not, it seems, unanimity (Osborne, *Int. Sugar J.*, 1923, 497; Stanĕk and Vondrák, *ibid.*, 1927, 225; Spengler and Brendel, *Brit. Chem. Abstr.*, 1927, *B*, 234; Eynon and Lane, *J. Soc. Chem. Ind.*, 1927, **46**, 177T). For chocolate products, corrections for volume of insoluble matter have been worked out and usefully tabulated by Fincke (*Z. Unters. Nahr. Genussm.*, 1925, **50**, 351; *Brit. Chem. Abstr.*, 1926, *B*, 252) and Jorgensen (*Ann. Falsif.*, 1925, **18**, 517; *Brit. Chem. Abstr.*, 1926, *B*, 212).

The correction for milk products has been established with precision by the Milk Products Sub Committee of the Society of Public Analysts (see section on "*Milk Products*," p. 261).

Adsorbent carbon for decolorising sucrose solutions must be used with caution, as Vašatko has found that decomposition of sugar can occur at temperatures above normal. There is a definite adsorption equilibrium between sucrose and carbon, which he has determined (*Z. Zuckerind. Czechoslov.*, 1927, **52**, 21, 129; *Brit. Chem. Abstr.*, 1928, *B*, 29).

In filtering solutions for polarisation, it is necessary to exercise care to prevent slight changes in concentration. Filter papers which contain more or less than about 7 per cent. of moisture have been shown to give up water to, or abstract it from, sugar solutions (Hardin and Zerban, *J. Ind. Eng. Chem.*, 1924, **16**, 1175; *Int. Sugar J.*, 1925, 53). Air-dry papers should be used, and a good portion of the filtrate rejected. Evaporation during filtration may be almost completely prevented by simply covering the funnel with a watch-glass (*Bur. of Stand. Circ.*, No. 44, "Polarimetry," 1918, 165).

Density and Refraction. The determination of density has always been a necessary auxiliary in the analysis of sugar products, especially in course of manufacture. There have been few developments recently in the comparatively simple apparatus used. Attention may be drawn to a sinker for the Mohr balance devised by Johansen (*Arch. Suikerind.*, 1925, **33**, 1008; *Int. Sugar J.*, 1925, 669), which is made by filling an electric bulb with mercury; with this, accuracy is increased, so that diluted solutions of molasses can be used.

The U.S. Bureau of Standards has published tables for correcting readings of saccharimeters made at other temperatures than the standard 20° C. (*Circular*, No. 19, 1924; *Int. Sugar J.*, 1925, 328), and for correcting Baumé readings to the same temperature (*Circular*, No. 295, 1926; *Brit. Chem. Abstr.*, 1926, *B*, 560). But as regards fundamental work on the density of sucrose solutions, nothing has been added to the classical data of Plato (1900) on which the above tables were based. There is scope for further work of this kind on other sugars, on sugar mixtures, and on the effects of impurities on the density of sugar solutions.

There is more progress to record with the refractometer, especially in its technical applications. The Zeiss sugar refractometer, an improved type of which was described by Prinsen-Geerligs (*Arch. Suikerind.*, 1923, **31**, 203; *Int. Sugar J.*, 1924, 52), and a still later one by Lowe (*Z. Ver. deut. Zuckerind.*, 1927, 690; *Brit. Chem. Abstr.*, 1928, *B*, 1), has set the mark for a special type of instrument adapted particularly for sugar readings. Zeiss have also succeeded in making refractometers which can be built into

the walls of vacuum and boiling pans, thus affording a means of following the sucrose content of a mother liquor directly in the pan. Lowe has published the tables necessary for the high temperatures at which this type of instrument is used (*Z. Ver. deut. Zuckerind.*, 1925, 360; *Int. Sugar J.*, 1925, 667).

The most recent development is a control refractometer by Bellingham and Stanley, which operates through a photo-electric cell and signal lamp.

Several portable refractometers are now available for the determination of dry substance in dilute saccharine solutions and juices (*Int. Sugar J.*, 1926, 223; 1928, 616).

By taking advantage of the precise determination of changes in refractive index possible by means of the interferometer, an attempt has been made by Horáček to range the refractive index alongside the optical rotation as a specific means for determining sucrose (*Z. Zuckerind. Czechoslov.*, 1926, **51**, 25; *Int. Sugar J.*, 1926, 677). The method is analogous to the Clerget method, being based on the increase in refractive index due to the hydrolysis of the sucrose (preferably by means of invertase); but it clearly requires further work before it can challenge polariscopic methods.

The most accurate data for the refractive index of sucrose solutions are due to Schönrock (*Int. Sugar J.*, 1911, 398), but he gives no figures for supersaturated solutions (above 66 per cent.). Such figures are often required, and the table then used is generally that of Main (*Int. Sugar J.*, 1907, **9**, 481), which appears to be accurate for high concentrations, though slightly in error for dilute syrups. There is an unfortunate small gap between the two sets of figures which ought to be filled up, since the sugar percentage in the neighbourhood of this point is often wanted with accuracy.

It is rather surprising that so little attention has been paid to the refractive index of solutions of other sugars, or to the influence of such sugars and of impurities on the refractive index of sucrose solutions; for the refractometer is perhaps the handiest and most rapid means of controlling the total soluble matter of many products whose main constituents are saccharine. Auerbach and

Borries (*Z. Unters. Nahr. Genüssm.*, 1922, **43**, 297 ; *J. Soc. Chem. Ind.*, 1922, 603A) have used it empirically for the dry matter of artificial honey, but were less successful with natural honey (*Z. Unters. Nahr. Genüssm.*, 1924, **48**, 272 ; *J. Soc. Chem. Ind.*, 1925, *B*, 848). The general problem should be attacked, however, more fundamentally by work upon the sugars concerned.

Electrical Conductivity.—Perhaps the greatest physical development in sugar work recently has been seen in the conductometric method for determining the mineral content of raw sugars, etc. The conductivity of a solution of a sugar is mainly due to the ions of the comparatively strongly dissociated mineral salts. Reichert, in 1889, first attempted to make use of this, but it was by Main (*Int. Sugar J.*, 1909, 334) and Lange (*Int. Sugar J.*, 1910, 423) that the method was put on a practical basis. Lange determined the conductivity of solutions of beet sugars whose ash content was found by the usual incineration method, and in this way obtained a correlation between the two which could be applied generally for converting conductivity into ash content.

The advisability of such a conversion now appears doubtful. The ratio between ash and conductance is not a constant for products of varying kind and origin, on account of differences in the nature of the mineral constituents ; so that a true ash figure cannot be obtained by using a constant factor. But the ash by incineration is itself quite an empirical measure of the mineral matter ; and the saddling of the conductivity determination on to an empirical ash seems to be only another way of paying tribute to convention. If the conductivity is simply accepted on its own merits, as a new and independent analytical criterion, it may be of more practical value in the end than the ash itself. It seems likely that it is a better measure of the melassigenic nature of the mineral substances ; and it certainly excludes insoluble and, in this sense, inert matter.

Moreover, by considering not the constancy, but the variations in the ratio between ash and conductivity, Spengler and Tödt (*Z. Ver. deut. Zuckerind.*, 1928, 1 ; *Brit. Chem. Abs.*, 1928, *B*, 422) have shown that a useful indication of the quality of the product can be obtained.

For convenience in making conductivity determinations, Todt (*Z. Ver. deut. Zuckerind.*, 1925, 429; *Int. Sugar J.*, 1925, 503) devised a simplified apparatus giving direct readings of the ash (based on Lange's tables) with an accuracy of about 0·2 per cent. of the ash when the latter was about 0·5 per cent. With larger proportions of ash he found dilution advisable. Nees used an apparatus which utilised the current from a lighting circuit, and incorporated a galvanometer and a dial-type Wheatstone bridge (*Ind. Eng. Chem.*, 1927, 225; *Int. Sugar J.*, 1927, 277). This apparatus was quite accurate enough for process control, and stood up to hard service. Zerban and Sattler's apparatus (*Facts about Sugar*, 1926, **21**, 1158; *Int. Sugar J.*, 1927, 390) comprises a slide-wire bridge, resistance box, hummer and telephone receiver, whilst Šandera (*Z. Zuckerind. Czechoslov.*, 1927, **51**, 205, 603; *Int. Sugar J.*, 1927, 280, 671) used lamp bulbs for balancing resistance in a bridge. His apparatus is now on the market. Still more recently he and Zimmermann have used the same optical balancing device in a special apparatus for the low conductivities of refined sugars. Other apparatus for these determinations includes a compact Leeds-Northrup bridge with galvanometer (*J. Sci. Instruments*, 1928, **5**, 296) and the "Salometer" (*Int. Sugar J.*, 1928, 205; 1929, 86), a bridge with hummer and telephones.

In view of what has been said above, the tables and other data published, giving the ash corresponding to various conductivities, are of only limited value (Lunden, *Int. Sugar J.*, 1925, 671). In experiments by Zerban and Sattler with raw sugars, the ash calculated from the conductivity was in most cases within 0·02 per cent. of the ash found by deducting 10 per cent. from the weight of sulphated ash (*Facts about Sugar*, 1926, **21**, 1158; *Int. Sugar J.*, 1927, 390). This may be more fortuitous than it sounds, since the deduction of 10 per cent. from the sulphated ash has been found to be much too low.

In later work, these authors have adapted their formulæ to syrups and molasses (*Int. Sugar J.*, 1929, 324).

Spengler and Todt have summarised the results from various sources for the ratios between conductance and ash (*Z. Ver. deut. Zuckerind.*, 1928, 1; *Brit. Chem. Abstr.*, 1928, *B*, 422).

There is no doubt that the simplicity and accuracy of the electrical method will result in a further rapid extension of its use.

Colour Measurement. Quite important advances have been made in the apparatus and methods of colorimetric measurement as applied to sugars. This is chiefly in relation to the actual colour of products themselves, as distinct from comparisons against standard solutions in micro-colorimetric tests.

Whilst the old colorimetry, typified in the Lovibond and Stammer instruments and their scale (cf. *Vitamins in Oils*, p. 84), compared the colours of solutions against arbitrary units, not always of suitable tone, the new seeks to define both depth and tone of colour in absolute terms, independent of the particular instrument employed. This is accomplished by measuring the amount of light absorbed at different wave-lengths throughout the spectrum; the shape of the absorption curve obtained in this way gives a picture of the colour-tone, and the amount of absorption measures the depth of colour. Solutions giving parallel absorption curves are thus simply different depths of the same colour. Characteristic curves, or variations in the curves, may be shown by particular constituents of a coloured solution, so that information may thus be obtained as to the nature of the colouring substances themselves. This has importance in the control of sugar manufacture or refining; Lundén (*Z. Ver. deut. Zuckerind.*, 1926, **76**, 780; *Chem. Abstr.*, 1927, 336) has in fact investigated the different types of colouring matter in factory juices, and, with the aid of the spectro-photometer, has followed them through the manufacturing processes.

One of the most refined of the new instruments is the polarisation spectrophotometer, in which the light source is divided into two beams. One of these passes through the solution to be examined, the other through a polariser, and later through a Nicol prism, by means of which it can be brought to the same intensity as that transmitted through the coloured solution. A dispersion prism is introduced into the system, enabling the field to be viewed at any required wave-length.

For more rapid work on a limited range of coloured solutions of

uniform type, the prism dispersion is replaced by suitable colour screens, so that the absorption of light of certain particular wave-lengths only is found. This suffices for many practical requirements.

The older types of colorimeter, such as the Duboscq, in which one solution is compared against an adjustable depth of a standard, have undergone improvements in many ways, especially since America, with supplies of European instruments cut off by the war, commenced to construct her own. To enable Duboscq-type colorimeters to be used for recording colour on the Stammer scale, Ritchie has proposed the use of ferric chloride solution as a comparison liquid (*Ind. Eng. Chem.*, 1927, **19**, 1289; *Brit. Chem. Abstr.*, 1927, *B*. 952); while Zert has sought to improve the readings of the Stammer instrument itself by replacing the plates by adjustable wedges containing 0·1 per cent. potassium dichromate solution (*Z. Zuckerind. Czechoslov.*, 1927, **52**, 57; *Chem. Abstr.*, 1928, 1707). On the other hand, Hoffmann has calculated the Stammer colour plates to their absorption in absolute units for various wave-lengths, as measured by the polarisation-photometer (*Z. Ver. dent. Zuckerind.*, 1926, **76**, 153; *Chem. Abstr.*, 1927, 1559); and Gibson, Harris, and Priest have similarly made a spectrophotometric analysis of a set of Lovibond glasses (*Bur. of Standards Sci. Paper*, No. 547, 1927) with a view to defining the Lovibond scale with respect to absolute terms.

As with polarimetric readings, there have been attempts to eliminate the judgment of the eye by employing photo-electric cells. Sandera uses a potassium cell, and attains an accuracy of 1 in 150; his apparatus can be used also for turbidities (*Z. Zuckerind. Czechoslov.*, 1928, **52**, 261; *Brit. Chem. Abstr.*, 1928, *B*. 344).

Fundamental work has been done by Peters and Phelps in America on the spectrophotometric analysis of sugar products. They have achieved a useful simplification in showing that the absorption for the particular wave-length $\lambda = 560\ \mu\mu$ can be converted directly into units of colouring matter of technical sugar products, relative to white light (*Sugar*, 1925, **27**, 333; *Int. Sugar J.*, 1925, 447, 616). Actual absorptions at this wave-

length are not conveniently determined in practice; but they can be readily interpolated from those for the wave-lengths $\lambda = 546$ and $\lambda = 578$, both of which are given by the mercury arc. Thus the use of the latter in conjunction with spectral filters avoids the necessity for an elaborate spectrophotometer (*Bur. of Standards J. Research*, 1929, **2**, 335; *Brit. Chem. Abstr.*, 1929, *B*, 336). The same workers have also gone thoroughly into the question of preparing sugar solutions for colorimetric analysis (*Bur. Standards Tech. Paper*, No. 338, 1927; *Brit. Chem. Abstr.*, 1927, *B*, 710).

These methods are chiefly valuable in control work and for products later to be used in the manufacture of other articles. What is required in the case of products ready for sale to the consumer is an article satisfactory in external appearance. Several instruments have been recently put forward for appraising such quality of appearance, among them the Eastman colorimeter (*Int. Sugar J.*, 1925, 613). Lundén has used the amount of fluorescence in the ultra-violet as a measure of the impurities in refined sugars, and claims to be able to distinguish cane and beet sugars in this way (*Zentr. Zuckerind.*, 1925, **33**, 1281; *Int. Sugar J.*, 1925, 614). It is doubtful how far this method deals with surface appearance, however, though it may be useful to the factory user of sugar.

Hydrogen Ion Concentration. Although the control of hydrogen ion concentration in the various stages of factory work has become a routine operation (Baleh, *Sugar*, 1925, **27**, 587; *Int. Sugar J.*, 1926, 223), and has even reached the stage of automatic control (Baleh and Paine, *Ind. Eng. Chem.*, 1928, 348; *Brit. Chem. Abstr.*, 1928, *B*, 461), there has been very little attention given to conditions of hydrogen ion concentration in analytical methods. There is no doubt, however, that as chemical methods become more closely investigated, and conditions are more stringently laid down, *p*H conditions will play a more important part. This will apply especially to the polarisation of impure products, and to colorimetric determinations, both of which are very much dependent on *p*H. That little has so far been done is possibly due to the fact that two of the main operations of sugar analysis,

the Fehling's reduction and the acid inversion of sucrose, are too drastic to come within the scope of refined *p*H conditions.

CHEMICAL METHODS

Copper Reduction. The overwhelming devotion to alkaline copper solutions in the chemical analysis of sugars, it seems likely, is due to two things: the commercial importance of sucrose, and its relative inability to reduce copper. These facts have influenced the whole development of chemical methods for sugars. Had sucrose been of insignificant importance to commerce, or, again, had it been a copper-reducing sugar, then more attention must have been paid to the other reactions of the common sugars. With things as they are, however, it was almost inevitable that the behaviour with copper should be seized upon and made the chemical discrimination *par excellence* between sucrose and the rest.

The history of copper methods, since Trommer's original discovery in 1841, has been a continual branching out into a wide diversity of detail which has now and then coagulated into an established method favoured by a particular group of workers. And with the gravimetric and indirect volumetric modifications the process still goes on. The dispersive tendency prevents the threads being drawn together into a universally accepted, if empirical method, upon which labour might usefully be spent in applying it to different sugars and mixtures. Only in the direct volumetric use of Fehling's solution, as we shall see, has a simplifying advance been made.

Part of the difficulty lies in the fact that sucrose is not altogether unaffected by the copper reagent. Gravimetric and indirect volumetric methods are characterised generally by the use of excess of alkaline copper, and owing to this, special care has to be exercised in order to minimise the action on sucrose, or at least to ensure that it occurs only to a definite extent which can be allowed for. This is difficult when the excess of copper may be very variable. Moreover, the reducing powers of the reducing sugars themselves are subject to slight variations, according to

the excess of reagent. Endless sets of tables have been published embracing the variations, when the treatment with the copper solution is carried out according to a particular procedure.

Gravimetric Copper Methods.– A thorough study of the sources of error to which the "precipitation" methods (*i.e.*, gravimetric and indirect volumetric) are liable, was made in 1921 by Quisumbing and Thomas (*J. Amer. Chem. Soc.*, 1921, **43**, 1503 : *Analyst*, 1922, **47**, 27). They found that it is better to carry out the reduction at a constant temperature below boiling-point, on account of the liability of the latter (whether of a water bath or of the solution itself) to vary. Moreover, the auto-reduction of Fehling's solution and its action on sucrose are much less at lower temperatures. As a convenient arbitrary procedure they adopted a heating period of thirty minutes at 80° C. For these conditions they worked out the best composition for the Fehling's solution, examined and found how to obviate oxidation of the cuprous oxide exposed to the surface, and then determined the reducing powers of different proportions of the various sugars, and the extent of the effect of sucrose when also present.

Their method is obviously capable of so much closer control than previous methods involving shorter periods of heating at the boiling point that it was given extensive trial. In general, their claims as to greater accuracy were substantiated, though their actual values for the reducing powers of the sugars could not always be reproduced. As with all other copper reduction methods, it still seems desirable that each analyst should determine for himself the reducing powers of the pure sugars with which he is concerned, under the exact conditions in use.

The details of the method are as follows :

Solutions.— (1) Crystals of c.p. copper sulphate are washed free from dust, etc., dissolved, the solution filtered, and diluted so that 500 c.c. contain 41·2 gm. of $CuSO_4 . 5H_2O$.

(2) To a solution of 173 grms. of crystallised Rochelle salt is added a solution containing 65 grms. of sodium hydroxide free from carbonate, and the mixture is diluted to 500 c.c. (The exact alkalinity of the sodium hydroxide solution should be established by titration.)

Procedure.— Twenty-five c.c. of each solution are measured into a 100-c.c. Pyrex or Bohemian glass beaker, having a diameter of about

9 cm.; 50 c.c. of sugar solution are added and mixed in by swirling, and the beaker is covered with a watch glass and immersed in a water bath kept at 80° C. After thirty minutes' digestion, the cuprous oxide is brought on to the asbestos mat of a Gooch crucible, washed as usual, and the copper determined by a suitable method. (There seems no reason why the precipitate should not with advantage be estimated, without separation from the excess of Fehling's solution, by one of the methods to be described later.)

The authors gave equations and tables for the weights of the several reducing sugars corresponding to various weights of copper, but, as already suggested, it is preferable for the analyst to determine the factors corresponding to his own conditions, as small details, such as type of water bath, depth of immersion of beaker, etc., can slightly modify the factors.

The weights of sugars to be taken for analysis are: Dextrose, lævulose, or invert sugar, 50 to 150 mgrms.; lactose and maltose, 100 to 300 mgrms. Sucrose should not be present in excess of the following amounts in association with the respective sugars: Dextrose, 400 mgrms.; lævulose or invert sugar, 200 mgrms. (twice this with 160 mgrms. or more of reducing sugar); lactose, 200 mgrms.; maltose, 100 mgrms. The temperature of the water bath should be controlled to within ± 1° C. for most purposes, and more closely for high accuracy.

Another careful study has been made by Jessen-Hansen (*Comptes rend. Trav. Lab. Carlsberg*, 1923, **15**, No. 3; 1925, **16**, No. 4; *J.C.S. Abstr.*, 1923, ii., 882; *Brit. Chem. Abstr.*, 1926, *B*, 294), who determined the amount of cuprous oxide deposited under standard conditions by pure sugars and by mixtures of them, and gave formulæ and tables for the several cases. But his procedure was hardly suitable for general work, as it required an atmosphere of hydrogen. Moreover, by carrying out the reduction in a boiling water-bath, he was left with some of the sources of error that Quisumbing and Thomas had eliminated.

The chief difficulty in copper reductions carried out at the boiling-point is an unevenness in boiling temperature, causing a corresponding fluctuation in the precipitation of cuprous oxide by any sucrose present. This has been partly overcome by Bruhns (*Zentr. Zuckerind.*, 1922, **30**, 1473; *J. Soc. Chem. Ind.*,

1923, 195A) and Pick (*Z. Zuckerind. Czechoslov.*, 1925, **49**, 211, etc.; *Int. Sugar J.*, 1925, 446), who found that superheating effects were partly responsible and could be eliminated by the addition of a little talc powder or nitric acid-extracted charcoal. Even then, however, there remains the effect of barometric pressure. It is not easy for others to standardise a boiling-point method so as to satisfy the chemist of Wyoming, for example, in whose laboratory water boils at 92·8° C. (*J. Assoc. Off. Agric. Chem.*, 1923, **6**, 336). This is extreme, but much smaller departures from the normal must be significant.

Indirect Volumetric Copper Methods.—For the determination of the precipitated cuprous oxide of the above and other precipitation methods, the usual procedure for long was to convert to cupric oxide (by ignition), or to copper (by reduction in hydrogen), or to dissolve and estimate iodometrically or by electrolysis. It is probable that all such methods, involving removal of the precipitate by filtration, tend to give erratic results, by reoxidation of the cuprous oxide during filtration and washing. Moreover, direct weighing of the copper or cupric oxide is liable to be inexact owing to impurities, organic and inorganic, carried down by the precipitate.

For these and other reasons, volumetric methods of determining the copper indirectly were introduced. The best-known processes of this kind were those of Maquenne and of Schoorl (*Int. Sugar J.*, 1916, 334), who estimated the excess of cupric salt by the usual iodometric titration. It was unnecessary to remove the cuprous oxide from the solution. There has been some controversy among Dutch chemists as to certain possible sources of error (such as time of cooling, time between acidification and titration, amount of iodide used, etc.), and it is evident that conditions must be carefully controlled for good results.

The Schoorl titrations consume rather large quantities of potassium iodide; and considerations of expense and the stringency of war conditions led Brühns (*Chem. Ztg.*, 1921, **45**, 486; *Analyst*, 1921, **46**, 330) to substitute potassium thiocyanate for the greater part of the iodide, with some success. The method has, however, not been free from criticism (Auerbach and Bodländer, *Z. angew.*

Chem., 1922, **35**, 631 ; *J. Soc. Chem. Ind.*, 1922, 991A). Errors may occur, according to Kunz, owing to slight variations in time of boiling. Brulus used the customary Herzfeld procedure of boiling for two minutes, and it is by no means easy to judge the exact time within as much as ten seconds. Further, Brühns' published tables have been called in question, which again emphasises the necessity for the analyst to establish his own standards, whatever process he uses.

Iodide was eliminated altogether by Fleury and Tavernier (*Bull. Soc. Chim.*, 1924, **35**, 794 ; *Chem. Abstr.*, 1924, 2854) who used a mixture of thiocyanate and thiosulphate. The excess of cupric salt was reduced to cuprous thiocyanate at the expense of the thiosulphate, and the excess of the latter was readily titrated with iodine.

These methods are subject to the disadvantages common to indirect methods generally ; but a new departure was made in 1921 when Shaffer and Hartmann devised an elegant method for the direct iodometric estimation of the cuprous oxide itself (*J. Biol. Chem.*, 1921, **45**, 349, 365 ; *J. Chem. Soc.*, 1921, **120**, ii., 417). The reaction involved is the reversible one :

$$2\,Cu^{++} + 4\,I^- \rightleftarrows 2\,Cu^{+} + 2\,I^- + I_2$$

In the ordinary titration of cupric salts (as in Schoorl's method) the reaction proceeds to completion to the right, since the iodine is removed from the solution by thiosulphate. Shaffer and Hartmann, by adding an alkali oxalate to the solution, reversed the direction, and were thus able to titrate cuprous oxide, even in presence of much excess of cupric salt.

The precipitation is carried out by Munson and Walker's method ; though any similar one, such as that of Quisumbing and Thomas, may be used. To the cooled mixture, 50 c.c. (or 25 c.c. if but little cuprous oxide is present) of iodide-iodate solution are added, followed by 15 to 17 c.c. of 5*N* sulphuric acid, added quickly. The solution is shaken gently until the cuprous oxide has dissolved, when 20 c.c. saturated potassium oxalate are added and the flask rotated until any cuprous iodide which has separated has completely dissolved. The solution is then titrated with 0·1*N* thiosulphate, with starch at the end-point. A blank test on the Fehling's solution is also made. The thiosulphate difference between the two titrations is calculated to

copper : 1 c.c. 0·1*N* thiosulphate = 6·36 mgrms. copper. The iodide-iodate solution consists of 60 grms. of potassium iodide, and 5·4 grms. of potassium iodate, with a little alkali, per litre, and should be accurately standardised against the thiosulphate.

An investigation of the conditions necessary for exact titrations of excess of cupric salt (as in Schoorl's method) was also made by Shaffer and Hartmann ; and both cuprous and cupric titrations were applied to the determination of small amounts of sugar in milk, urine and blood. For this purpose they used a combined reagent incorporating the iodide mixture with a copper reagent : but it has since been found better to omit the iodide from this mixed solution, and add it after reduction of the copper (De Long). A good account of the method is given in *Thorpe's Dictionary* (1926, Vol. VI., 487).

Pick (*Z. Zuckerind. Czechoslov.*, 1925, **49**, 251 ; *Int. Sugar J.*, 1925, 501) investigated the method in some detail, and drew attention to the necessity for the absence from the Fehling's solution of ferric iron and of nitrite. Copper sulphate could be freed from iron by recrystallising from hydrochloric acid solution. It was also necessary to correct for the influence of the iodine on the solution being analysed.

A further improvement by Blanchetière consists in the substitution of oxalic acid for the sulphuric acid, so that no iodine is liberated from the iodide, even when a large excess of cupric salt is present (*J. Soc. Chem. Ind.*, 1924, 772A).

When only very small amounts of cuprous oxide are concerned, the direct oxidation with iodine can be effected without the addition of oxalate. Kraisy used the method in this way in determining the cuprous oxide precipitated by highly purified sucrose from his special "neutral" Fehling's solution, which was a weak preparation made with sodium carbonate instead of hydroxide (*Z. Ver. deut. Zuckerind.*, 1921, 123 ; *J. Soc. Chem. Ind.*, 1921, 315A).

The movement in favour of methods not requiring the separation of the cuprous oxide has made the older Bertrand process (*J. Soc. Chem. Ind.*, 1907, 60) seem less desirable. This depends on the solution of the precipitate in acid ferric sulphate or iron alum, and

titration of the reduced iron with permanganate. Amick, moreover, has now shown that tartrate adsorbed by the asbestos or filter paper is removed when the cuprous oxide is dissolved by the iron solution, and then introduces errors into the titration (*J. Phys. Chem.*, 1927, **31**, 1441 ; *Brit. Chem. Abstr.*, 1927, *A*, 1213). Amick's results afford equally valid objections to certain methods which have been suggested for the treatment of the cuprous oxide with acidified permanganate, followed by determination of the excess of the latter (Bisson and Sewell, *J. Assoc. Off. Agric. Chem.*, 1927, **10**, 120 ; *Analyst*, 1927, **52**, 289). For routine work, however, where very exact results are not required and cheapness of reagents is a desideratum, the latter method may be a useful alternative to Bertrand's.

By substituting bromate for permanganate, Mislowitzer has found that the titration can be made in presence of the excess Fehling's solution (*Biochem. Z.*, 1926, **168**, 217 ; *Brit. Chem. Abstr.*, 1926, *A*, 442). There is, however, no convenient colour change which can be used as end-point, and this is ascertained potentiometrically, a simple apparatus being described for this purpose. Potentiometric methods, however, may perhaps be more simply applied to a direct volumetric use of Fehling's solution itself, as will be seen later.

Direct Volumetric Copper Methods. All the volumetric methods so far considered, depending on the use of excess of copper solution, have to be clearly distinguished from the simple direct volumetric use of Fehling's solution. This involves a reduction of the whole of the copper present, and titrations are conducted in such a way that most of it is rapidly thrown down as cuprous oxide before ebullition commences. The action on any sucrose present is accordingly lessened, and such variable influences as the atmospheric pressure are of less consequence.

Internal Indicators.—Until recently the necessity for the use of outside indicators and the tendency to back oxidation whilst the outside test was being made, had prevented the general adoption of the method for exact purposes. The introduction by Lane and Eynon (*J. Soc. Chem. Ind.*, 1923, **42**, 32T) of the use of methylene blue as an internal indicator was an advance of the first

order. So great has been the gain, in fact, that R. F. Jackson recently stated (*J. Assoc. Off. Agric. Chem.*, 1929, **12**, 166) that "the method is, on account of its convenience, accuracy, and rapidity, largely displacing the gravimetric methods for reducing sugar." And so the diversity of gravimetric procedure, the mountains of tables embodying the personal fancies of different workers, the added disadvantages of indirect volumetric methods, are all now challenged by the simplicity of a direct titration. When a fraction of the labour expended on the older methods has been devoted to the study of the mutual influence of mixed sugars in the Lane and Eynon titration, the process should attain all but the utmost that can be expected from a copper reduction method.

The Fehling's solution used by the authors is the usual Soxhlet modification containing 34·64 grms. of pure crystallised copper sulphate per litre of mixed solution. (From Quisumbing and Thomas's work it might be well to pay some attention to securing correct alkalinity, especially by avoiding the inclusion of carbonate.) The indicator is a 1 per cent. solution of methylene blue in water.

For the titration, 10 c.c. or 25 c.c. of Fehling's solution are accurately measured into a conical flask of about 300 c.c. capacity. From a burette is added almost the full amount of sugar solution required to reduce all the copper (ascertained by a preliminary titration), and the flask and contents are heated over a wire gauze, preferably with a white asbestos centre. After boiling commences, it is maintained moderately for two minutes, when 3 to 5 drops of the indicator are added, and the titration is continued so that it is just complete in a total boiling time of three minutes. It is unnecessary, and inadvisable, to remove the flask from the gauze at any stage of the titration; the end-point is clearly indicated by the disappearance of the colour of the methylene blue, the whole mixture becoming bright red or orange in colour. A burette with a rubber tube and pinchcock or glass bead should be used, as glass taps are liable to become jammed by being held over the boiling liquid.

The preliminary titration is carried out by adding the sugar solution in fairly large portions at fifteen-second intervals until, from the colour of the mixture, the copper appears to be nearly all reduced; then boiling for a minute or two, adding the indicator, and completing the titration with smaller additions of sugar solution. An operator with experience can carry out this preliminary titration so as to obtain results almost as accurate for many purposes as by the standard titration already described.

The method, he considers, has promise, but involves tedious calculations in order to apply a correction for the dextrose reduction in unknown mixtures.

For the determination of reducing sugars when large amounts of sucrose are also present, as in testing refined sucrose, various modified copper reagents have been proposed. Schoorl has revived the use of Luff's copper citrate-carbonate mixture, its action on sucrose being much less than that of Fehling's solution (*Chem. Weekbl.*, 1925, **22**, 132; *J. Soc. Chem. Ind.*, 1925, 329A, *Z. Unters. Lebensm.*, 1929, **57**, 566; *Brit. Chem. Abstr.*, 1929, *B*, 952); whilst Ofner has proposed a copper tartrate solution with its alkalinity due to sodium carbonate and phosphate, which, it is claimed, permits the estimation of as little as 0·01 per cent. of invert sugar in refined sucrose (*Z. Zuckerind. Czechoslov.*, 1925, **49**, 279; 1929, **53**, 728; *Int. Sugar J.*, 1925, 668; *Brit. Chem. Abstr.*, 1929, *B*, 832); and Beyersdorfer has obtained good results with Ost's solution (*Z. Ver. deut. Zuckerind.*, 1919, 403; *J. Soc. Chem. Ind.*, 1920, 126A).

Reference may be made again in this connection to Kraisy's method (p. 26) for traces of invert sugar in specially refined sucrose.

Interfering Non-sugar Substances in Copper Methods. Most sugar determinations are carried out on more or less impure products, and copper reduction methods are liable to be influenced by some of the non-sugar substances present, or introduced by the clarification. Knowledge is continually extending as to how this interference can be avoided.

That the excess of basic lead acetate necessary in clarifications can cause loss of reducing sugar has been long known. The trouble appears to be due to the fact that lead, in slightly alkaline solutions, forms insoluble compounds with levulose. According to Harris (*J. Ind. Eng. Chem.*, 1921, 925; *Int. Sugar J.*, 1921, 701), if the excess of lead is removed by adding a suitable acid de-leading agent (such as oxalic acid) before filtering off the lead precipitate, no loss of reducing power occurs. On the other hand, Englis and Tsang (*J. Amer. Chem. Soc.*, 1922, **44**, 865; *Analyst*, 1922, **47**, 301) consider that the loss of levulose is due to occlusion,

and that it is relatively high when oxalates are used for de-leading, but negligible with phosphates.

In addition to lead, it is necessary to remove the alkaline earths if present, since they can introduce considerable errors into both gravimetric and volumetric copper determinations, especially of lactose (Eynon and Lane, *J. Soc. Chem. Ind.*, 1923, 143T). Their complete removal, along with excess of lead, can be effected best with oxalate (*J. Soc. Chem. Ind.*, 1923, 463T).

The interference of various nitrogenous substances was investigated by Rosenthaler (*Pharm. Zentr.*, 1925, **66**, 517; *J. Soc. Chem. Ind.*, 1925, 732A), who found large errors possible with urine and uric acid; and by Harlesová (*Z. Zuckerind. Czechoslov.*, 1929, **54**, 1; *Brit. Chem. Abstr.*, 1930, *B*, 76).

Borates, which are sometimes present in biological material, have been found to inhibit the reduction of alkaline, but not acid copper solutions (Levy and Doisy, *J. Biol. Chem.*, 1928, **77**, 733; *Brit. Chem. Abstr.*, 1928, *A*, 741). The interference of cyanides has been studied by Hérissey and Chalmeta (*J. Pharm. Chem.*, 1928, **8**, 393; *Analyst*, 1929, **54**, 43).

Other Reduction Methods.—Although copper solutions have been regarded as specially suitable oxidising agents for sugars, use has at various times been made of other common reagents, though until lately none of them has had much vogue. In the recent period, Quisumbing has worked out a method for the use of permanganate, by heating with the latter for a definite time and determining the excess with oxalic acid (*Philippine J. Sci.*, 1920, **16**, 581; *Analyst*, 1921, **46**, 58). But this method is no less empirical than copper methods, and appears to have no special advantages over the latter.

More success has attended the use of potassium ferricyanide, the reduction of which compound by sugars was first observed by Gentele (1861). It has the advantage over copper that the reduction product is not re-oxidised so readily by atmospheric or dissolved oxygen. Jonescu and Vargolici have employed the reaction for determining sugars by titrating the sugar solution to the boiling alkaline ferricyanide solution, and noting the passing of the colour (*Bull. Soc. chim. Roman.*, 1920, **2**, 38, 102; *Analyst*,

1920, **45**, 339; 1921, **101**, 1051. With coloured solutions the end-point is obtained by means of picric acid. For determinations of lactose in milk, for instance, it is convenient, therefore, to clarify with a mixture of acetic and picric acids. When the sugar to be determined is much less than 0·5 per cent., it is better to boil with excess of the reagent and titrate the ferrocyanide formed with permanganate. In either case, the ferricyanide or permanganate is standardised against known sugar solutions of approximately the concentration of the unknown.

Alkaline Iodine Reduction.—Most of the reduction methods that have been discussed draw very much the same line between the "reducing" and non-reducing sugars (mainly sucrose). In this sense, the former are sugars with either a free aldehyde or a free ketone group. A method which cuts across this classification, and subdivides the reducing group, should find obvious applications where mixtures are concerned. This desirable end can be attained by utilising the oxidising properties of alkaline iodine solutions, which appear to contain hypoiodite as the active constituent. Such solutions, at ordinary temperatures, convert the aldose sugars with great facility into the corresponding monobasic acids, provided that the iodine is in sufficient excess, and the degree of alkalinity is suitable. The type of the reaction is

$$C_5H_{11}O_5\ CHO + I_2 + 3NaOH = C_5H_{11}O_5\ COONa + 2NaI + 2H_2O$$

Thus 1 grm. of a hexose sugar requires 1·110 grm. of iodine for oxidation, while 1 grm. of maltose or lactose (as hydrate) requires exactly half that quantity.

Besides the main reaction, there seems to be a subsidiary action on the alcoholic groups of the sugar, which is so small as to be usually negligible. Ketose sugars (in particular, lævulose), do not undergo the main oxidation, but appear to be more exposed to the attack on the alcoholic groups. The latter is, however, not considerable, and allowance can be made for it.

Not only is this reaction a useful help in analysing mixed sugar products, but the simplicity and convenience of the method recommend it for replacement of other reduction methods in the

determination of single sugars, as for lactose in milk products. The end-point, being iodometric, is very sharp, and the process is suitable for quite small quantities of sugar; it can, in fact, be used as a micro-method (Perrier, *J. Pharm. Chim.*, 1920, **22,** 337; *Analyst*, 1921, **46,** 11. Macleod and Robison, *Biochem. J.*, 1929, **23,** 517).

Moreover, the oxidation is carried out at ordinary temperatures, and is thus altogether more delicate than the drastic heating with the strongly alkaline solutions of the copper methods. The action of the latter on the sugar molecule is too violent to be anything but empirical; whilst in the iodine oxidation the reaction is stoichiometrical, so that its progress can be checked and conditions adjusted so as to favour a straightforward course. In other words, it is possible to know just where the reaction should end. This also ensures that the analyst is not burdened with new and voluminous sets of tables whenever a slight modification in the technique is introduced, since such modifications have to conform to the proper course of the reaction.

Owing to the sensitive nature of the oxidation, non-sugar substances, such as proteins, alcohols, etc., more readily interfere than with copper. This, however, does not seem to be an objection of much weight. When the method receives study in anything like the detail of the copper methods, means should readily be found for dealing with interfering substances.

The method was first put forward by Romijn (*Z. anal. Chem.* 1897, **36,** 349; *J. Soc. Chem. Ind.*, 1897, 765), and it is remarkable that his discovery should have lain dormant for so long. It is even more surprising that an apparently independent discovery of the method by Bland and Lloyd (*J. Soc. Chem. Ind.*, 1914, **33,** 948), almost in the form later shown to give the best results, remained completely neglected. This is, perhaps, again because of the importance of sucrose, and the convenience of grouping the common non-sucrose sugars together as "reducing." The growing importance of sugar determinations for biological purposes, however, in which dextrose is the sugar of chief interest, and a certain desire for emphasising the distinction between dextrose and lævulose, led to a revival of the method in 1917 by Bougault

(*Compt. rend.*, 1917, **164**, 1008; *Analyst*, 1917, **42**, 307), and in 1918 by Willstatter and Schudel (*Ber.*, 1918, **51**, 780; *Analyst*, 1918, **43**, 416).

Complete oxidation with Romijn's solution of iodine and borax required from sixteen to twenty-two hours, and Bougault improved upon this by using sodium carbonate instead of borax, shortening the time to half an hour. Willstatter and Schudel still further reduced the time, without loss of accuracy, by using sodium hydroxide in limited amount, equal to one and a half times the equivalent of the iodine. To a solution of 0·1 grm. of sugar in 10 c.c. of water they added 20 c.c. of 0·1*N* iodine and 30 c.c. of 0·1*N* sodium hydroxide, allowed the mixture to stand for fifteen to twenty minutes, and then acidified with dilute acid, and titrated the excess of iodine with 0·05*N* thiosulphate.

The method gathered impetus in this form, and various workers took a hand in shaping it for particular purposes: Judd (*Biochem. J.*, 1920, **14**, 255; *Analyst*, 1920, **45**, 224) for plant juices; Baker and Hulton (*Biochem. J.*, 1920, **14**, 754) (verifying the quantitative nature of the oxidation of the other aldose sugars, maltose and lactose, etc.) for starch conversion products; and Behre (*Z. Unters. Nahr. Genussm.*, 1921, **41**, 226; *Analyst*, 1921, **46**, 368) for sucrose and dextrin in mixed products by determining the dextrose formed from them by hydrolysis. A similar application to mixed products is due to Kolthoff (*Z. Unters. Nahr. Genüssm.*, 1923, **45**, 141; *Analyst*, 1923, **48**, 386).

In addition to working out more closely the conditions necessary for the estimation of lactose, invert sugar, and sucrose (by inversion), Hinton and Macara (*Analyst*, 1924, **49**, 2) investigated the extent of the oxidation of the relatively inert sugars, lævulose and sucrose. They emphasised the importance of the control of time and temperature conditions when these sugars were present, so that an allowance could be made for the precise amount of their interference. The use of weaker alkalis than sodium hydroxide was not found advantageous.

The iodine reduction method has peculiar importance in cases where the content of dextrose is required, as distinct from lævulose. For this purpose it may be used in combination with a copper

reduction method giving the total reducing sugar (dextrose and lævulose). Thus for comparing the proportions of the two sugars in natural products such as plant juices and honey, and for detecting the admixture of the latter with artificial invert sugar (in which the proportions are different) it has received some attention (Auerbach and Bodländer, *Z. Unters. Nahr. Genussm.* 1924, **47**, 233; *Analyst*, 1924, 389. Schnette, *J. Assoc. Off. Agric. Chem.*, 1929, **12**, 151). In such products as artificial invert sugar itself, where condensation products of lævulose are liable to interfere with estimations of sucrose by the usual methods, the iodine method, which ignores the interfering lævulose, has a special value (Behre, Düring, and Ehrecke, *Z. Unters Nahr. Genüssm.*, 1921, **42**, 242; *J. Soc. Chem. Ind.*, 1922, 71A).

After oxidising (and, if necessary, determining) other reducing sugars (aldoses) with iodine, lævulose can be separately determined by copper reduction (Kolthoff, *Chem. Weekblad*, 1922, **19**, 1; *Analyst*, 1922, **47**, 301). The use of the iodometric oxidation qualitatively in this way really establishes a new principle in the analysis of products containing lævulose or sucrose (or both), which has been further developed by Kruisheer (*Z. Unters. Lebenam.*, 1929, **58**, 268) and made the central feature of a scheme of analysis of mixed sugar products containing starch glucose, etc. This reduction in stages has also been used for such determinations as that of honey in cakes (Mees, *Chem. Weekbl.*, 1928, **25**, 674; *Brit. Chem. Abstr.*, 1929, *B*, 109).

Various modifications of the iodine method that have been proposed consist mainly in altering the degree of alkalinity of the reacting solution, with a view to suppressing the secondary action on the sugars. In some cases, especially with alkali hydroxide, it seems that the oxidation of the aldose group itself is not quite complete when the whole of the necessary alkali is added immediately. Goebel has found that for complete oxidation the alkali should be added over a period of two minutes, so as to prevent the formation of iodate at the expense of hypoiodite, whilst at the same time avoiding the formation of an unoxidisable ketone from a small fraction of the aldose (*J. Biol. Chem.*, 1927, **72**, 801; *Brit. Chem. Abstr.*, 1927, *A*, 648).

The necessary gradual addition of alkali is achieved automatically in the latest form of the iodine method, due to Hinton and Macara (*Analyst*, 1927, **52**, 668). They use a mixture of chloramine-T and potassium iodide as the oxidising agent. Hypoiodite is formed in the solution by interaction of the iodide and chloramine-T, whilst the necessary alkalinity is progressively provided as the reaction proceeds, by hydrolysis of part of the hypoiodite, so that at no time is there a great excess. This probably explains the slower speed of the reaction, which renders it more easily controlled, and the smaller subsidiary attack on lævulose and sucrose. The chloramine-T modification was designed particularly for exact determinations of sugars in milk and milk products; milk serum clarified with phosphotungstic acid contains no substances other than sugar which are oxidisable under the specified conditions. Small corrections are required, in exact work, for the effect of concentrations of sugars in the solutions titrated, and for the effect of sucrose in the lactose titration; these are expressed in the form of convenient tables in the original paper, together with the details of the oxidation and titrations.

Colorimetric Methods. Most of the new colorimetric methods have been devised for microbiological tests, and are described later. One more generally useful, however, is that depending on the reduction of alkaline picrate solutions to a reddish-brown colour, which can be compared with the colour from a known amount of the appropriate reducing sugar. It is particularly useful where the highest precision is not required, or can be sacrificed to rapidity of working, as in routine analyses of botanical or dairy products. A large excess of sucrose seems to interfere.

The method seems to have been first described by Dehn and Hartmann (*J. Amer. Chem. Soc.*, 1914, **36**, 403; *Analyst*, 1914, **39**, 180), for lactose in milk particularly. They claimed an accuracy of ± 1 per cent. Later workers have recommended it, in various modifications, for rapidly determining small proportions of sugars in miscellaneous foodstuffs. References may be made to Coe and Bidwell (*J. Assoc. Off. Agric. Chem.*, 1924, **7**, 297; *Int. Sugar J.*, 1924, 279); Willaman and Davison (*J. Agric. Research*, 1924, **28**, 479; *Analyst*, 1924, **49**, 591); Thomas and

Dutcher, for plant extracts (*J. Amer. Chem. Soc.*, 1924, **46**, 1662; *Int. Sugar J.*, 1924, 610); and Bierman and Doan, for milk products (*J. Dairy Sci.*, 1924, **7**, 381; *J. Soc. Chem. Ind.*, 1925, *B*, 223).

Sucrose may be determined by inversion, which may be effected by the picric acid itself, in which case the solution is not made alkaline until after the inversion treatment.

Schachkeldian (*Brit. Chem. Abstr.*, 1929, *A*, 298) has recently found that chlorine ions interfere with the colour production, and render special chloride-containing standards necessary.

A rather similar method is Summer's dinitrosalicylate test, which Kolthoff adapted for traces of invert sugar in refined sucrose, and found preferable to volumetric methods (*Archief Suikerind.*, 1922, **30**, 867; *Int. Sugar J.*, 1923, 662).

Miscellaneous Chemical Methods.—There have been attempts in various directions to make use of other chemical properties of the sugars, but none has so far had the general vogue of the reducing methods. Some of them deserve further study, especially where they can be used in helping to disentangle the components of reducing sugar mixtures.

A curious partiality has been displayed on the Continent for methods depending on the selective destruction of the sugars by heating. Dubrunfaut long ago discovered that most sugars except sucrose are decomposed by heating their solutions with alkali. Later workers, notably Jolles (*Int. Sugar J.*, 1911, 109), developed this idea, and have improved it by substituting alkaline earth hydroxides for the alkali (Behre and During, *Z. Unters. Nahr. Genussm.*, 1922, **44**, 65; *Analyst*, 1922, **47**, 478). The unattacked sucrose is determined by one of the usual methods. The process has been successfully used for sucrose in honey or invert sugar mixtures, and in chocolate and milk products (Fincke, *Z. Unters. Nahr. Genussm.*, 1925, **50**, 351; *Brit. Chem. Abstr.*, 1926, *B*, 252).

On the other hand, heating a sugar solution with moderately strong hydrochloric acid at the boiling point for some hours, according to Lucius (*Z. Unters. Nahr. Genussm.*, 1923, **46**, 94; *Analyst*, 1923, **48**, 607), destroys lævulose, and permits of the

determination of dextrose in mixtures of the two. The results can only be approximate, however, since Davis and Daish (*Analyst*, 1913, **38**, 504) have shown that dextrose is itself to some extent destroyed under these conditions.

A variation of this procedure is due to Riffart and Pyriki (*Z. Unters. Nahr. Genussm.*, 1924, **48**, 197; *Analyst*, 1925, **50**, 26), who heat with 70 per cent. sulphuric acid at 55° C. (after removal of fat, albumin, etc.) and measure the colour produced, which is proportional to the concentration of lævulose. This was found suitable for such products as condensed milk, biscuits, chocolate, and beer, though, again, the results cannot be very precise.

Attempts have been made to use the osazones of the sugars as a means of determination. Knecht and Hibbert, by employing a considerable excess of phenylhydrazine, were able to convert the sugar quantitatively into the osazone. The latter, in boiling solution, was then determined by treating with excess of titanous chloride, and titrating the excess with a solution of crystal scarlet (*J. Chem. Soc.*, 1924, **125**, 2009). The reaction has the advantage of being stoichiometric, and distinctly merits further trial.

Pentoses and Pentosans. Several attempts may be recorded at improving the classical method of Tollens for determining pentoses and pentosans by converting into furfural and distilling the latter.

Tollens precipitated the furfural in the distillate with phloroglucinol, after first using and abandoning phenylhydrazine. There has been a return to phenylhydrazine by Menaul and Dowell (*J. Ind. Eng. Chem.*, 1919, 1024; *Analyst*, 1920, 23), who estimated the excess of phenylhydrazine by measuring the nitrogen evolved from it; and by Ling and Nanji, who determined the excess iodometrically (*Biochem. J.*, 1921, **15**, 466; *Analyst*, 1921, **46**, 512). A new method was introduced by Pervier and Gortner, in titrating the distillate with potassium bromate, with an electrometric end-point (*J. Ind. Eng. Chem.*, 1923, **15**, 1255; *Analyst*, 1924, **49**, 106). Methylpentoses and methylpentosans, however, interfere, but if unmixed with pentose may also be determined by this method. In place of the electrometric end-point, excess of

bromate may be used and titrated back, according to Powell and Whittaker (*J. Chem. Soc. Abstr.*, 1924, ii., 354).

The steam distillation, substituted by Jolles (*J. Soc. Chem. Ind.*, 1906, 201) for the ordinary Tollens distillation, so as to keep the concentration of the acid constant, is slow; but this was overcome by Macheleidt by a double distillation (*Woch. Brau.*, 1922, **39**, 90; *J. Soc. Chem. Ind.*, 1924, *B*, 116). The concentration of acid may better be kept constant, according to Kullgren and Tydén (*Handl. Ing. Vetenskaps-Akad. Stockholm*, 1929, No. 94; *Brit. Chem. Abstr.*, 1929, *A*, 1278), by using a constant-boiling mixture of 13·15 per cent. hydrochloric acid saturated with sodium chloride.

The determination of pentoses and pentosans in mixed products can hardly yet be considered very reliable. Klingstedt, among others, has critically studied the subject, showing how hexoses and even cellulose may interfere (*Z. anal. Chem.*, 1925, **66**, 129; *Analyst*, 1925, **50**, 416). By adopting certain precautions, a fair estimate of the pentose may be obtained. According to Fukai, the methyl and hydroxymethyl derivatives of furfural, which are often jointly precipitated with the latter by phloroglucinol, can be separated from it by taking advantage of the different solubilities of the phlorogluecides in acetone (*Chem. Abstr.*, 1929, **23**, 1081).

An adaptation of the distillation method for microchemical purposes has been described by Youngburg (*J. Biol. Chem.*, 1927, **73**, 599; *Analyst*, 1927, **52**, 484); whilst the colour test for pentoses with Bial's reagent (orcinol and ferric chloride) has been used by Scheff for a spectrophotometric determination (*Biochem. Z.*, 1924, **147**, 94; *J. Soc. Chem. Ind.*, 1924, *B*, 724).

BIOCHEMICAL METHODS

Purely biological methods have been little used in sugar analysis, and are quite unsuitable for quantitative work. The method evolved by Castellani and Taylor (*J. Trop. Med.*, 1922, **25**, 41; *J. Chem. Soc.*, 1923, **124**, ii., 265), for characterising various sugars by means of their reactions with bacteria and moulds, shows how far

it has been possible to go in this direction. On the other hand, the use of biological agents in combination with physical or chemical methods has been fruitful in providing valuable selective determinations of particular sugars. This is because it is possible to find suitable organisms or enzymes acting specifically on certain sugars. We have already seen with what success the enzyme invertase has been applied in the polarimetric determination of sucrose. This is by far the best-known example of a biophysical method. The diastatic conversion of starch is, too, well established as a biochemical method and is reliable on its biological side. It suffers chemically only in so far as the methods for determining the reducing sugars formed from the starch lack accuracy, and will improve as they are improved.

The discovery by Bourquelot and Bridel (*Compt. rend.*, 1912, **155**, 86, 319; 1920, **170**, 631; *Analyst*, 1920, **45**, 175) that the hydrolysis of glucosides by emulsin is reversible in the presence of a sufficient concentration of methyl alcohol, led them to propose a novel method for determining dextrose in plants, by following the decrease in reducing power as the alcoholysis proceeded. Dextrose was distinguished from other sugars in fruits in this way by Arnold (*Bull. Soc. Chim. Biol.*, 1921, **3**, 547; *J. Chem. Soc.*, 1922, **122**, i., 311). Later, the method has been extended to the detection of galactose in mixtures with arabinose (Bridel and Charpentier, *J. Pharm. Chim.*, 1924, **30**, 33; *Analyst*, 1924, **49**, 445).

Until the period under review, the principal application of fermentative methods was in the complete removal of fermentable sugars from mixed products, so that starch products could be determined. The residue of unfermentable material would be chiefly starch, dextrins, and gums. A development of this procedure for the resolution of complex mixtures was proposed by Nanji and Beazeley (*J. Soc. Chem. Ind.*, 1926, **45**, 220t), who, in addition to a combination of the fermentation processes with copper reduction, brought in the newer iodine reduction, and so were able to detach lævulose as a separately determinable constituent. The principles of their methods are sound, but the inversion process, and the mode of correcting for the action

of iodine on lævulose, are not altogether satisfactory. Their system involves the following steps : (1) Copper-reducing power, gives dextrose, lævulose, maltose, etc., and the slightly reducing dextrins. (2) Copper-reducing power after inversion with 10 per cent. citric acid, gives same sugars plus inverted sucrose. (3) Iodine reduction, gives all copper-reducing substances except lævulose. (4) Polarisation, in conjunction with the other results, gives dextrose and maltose separately. (5) Specific gravity of solution before and after fermentation with Frohberg yeast (alcohol being removed by evaporation), gives dextrin. (6) Ash and protein determined in the usual way.

The newer fermentation processes will aim at distinguishing between the fermentation characteristics of the different sugars by means of specially selected strains of yeasts, each acting only on certain components of sugar mixtures. In this way there are possibilities quite outside the reach of chemical methods.

A distinct advance along these lines was made by McLachlan (*Analyst*, 1928, **53,** 583), who used three types of yeast in pure culture in analysing malt extract and commercial glucose. He firstly made use of the discovery of Davis and Daish (*J. Agric. Sci.*, 1913, **5,** 437 ; *Analyst*, 1913, **38,** 504) that *S. exiguus*, whilst fermenting dextrose (and lævulose if present), leaves maltose unattacked. Then, further, he conducted fermentations with *S. Frohberg* and *S. Saaz* (obtained from the National Collection of Type Cultures), the former fermenting maltose as well as dextrose and lævulose, whilst the latter removed all fermentable sugars. A blank solution was also used, and in this and the fermented solutions (after removal of alcohol) the specific gravity was determined. The differences between the various soluble solids figures so obtained gave : (1) Dextrose (and any lævulose), (2) maltose, (3) other fermentable sugars, and (4) dextrin ; with good approximate results. Confirmatory results were obtained from the optical rotations or the reducing powers. In this connection a specific rotation of 180 was found applicable to the residual dextrins ; but the changes in optical rotation, while confirming the maltose figures, only agreed for the dextrose if the latter were assumed to include a small amount of lævulose (not distinguishable

by the *S. exiguus*). The lævulose could, no doubt, be separately determined, if required, by adding an iodine reduction. Sucrose, if present, would be determined in the usual way by inversion, preferably with invertase.

The routine method suggested by McLachlan is the following: Eight tubes containing 50 c.c. each of a 10 per cent. solution of sample are sterilised in the steam steriliser on three successive days with rapid heating on the first day to destroy any diastase. Pairs of tubes are inoculated with *S. exiguus*, *S. Frohberg* and *S. Saaz*, and two are kept as a blank. All are incubated at 26° for fourteen days, with rotation of the tubes on the fourth or fifth day. After fermentation, one tube of each pair is emptied and rinsed out each into a 150 c.c. beaker, and the solutions are evaporated to about 15 c.c., cooled, made up to 50 c.c. and the gravities determined. The other tubes may be used for verification. The differences between the several results may be calculated to the various constituents in the way already mentioned.

Amongst other recent proposals for the specific use of biological agents is that of Müller (*Biochem. Z.*, 1929, **205**, 111; *Brit. Chem. Abstr.*, 1929, *A*, 470), who finds that an enzyme from the press-juice of certain moulds oxidises dextrose, but not lævulose or most other sugars, to the monobasic acid. Maltose, even in concentrated solutions, may be completely hydrolysed to dextrose, and so determined, by dried mycelium powder from *Mucor Boulard*, according to Colin (*Bull. Soc. Chim.*, 1926, **39**, 1481; *Brit. Chem. Abstr.*, 1926, *A*, 1229).

Snethlage proposed to determine lactose in extracts from bread by fermenting with *S. cerevisia* at 30°, thereby destroying all other sugars (*Chem. Weekbl.*, 1926, **23**, 578; *Brit. Chem. Abstr.*, 1927, *B*, 122).

MIXED SUGAR ANALYSES

There is, of course, nothing new in the combination of polarimetric and chemical methods for the purpose of indirectly distinguishing the components of sugar mixtures; but the placing of the individual analytical operations on a basis of greater accuracy, in the various ways that have been described, must have a doubly favourable effect on such mixed sugar analyses.

In one or two directions, owing to new chemical and biological methods, especially the direct iodine oxidation, it is now possible to separate the components more completely than could before be done.

The most valuable recent work in this direction has been done by Kruisheer (*Z. Unters. Lebensm.*, 1929, **58**, 261). Reference has already been made to his application of the iodimetric oxidation of aldoses for the purpose of subsequently obtaining direct determination of lævulose by copper reduction. In addition, he has worked out a precise method for the analysis of mixed sugar products (especially those containing starch, sugar and glucose), which depends on determining the total reduction and the lævulose reduction, (1) before inversion, (2) after "weak" inversion, and (3) after "strong" inversion. For determining copper reductions, Kruisheer used the carbonate-alkaline copper solution of Luff, revived as already mentioned by Schoorl. This has a negligible action on sucrose and on certain decomposition products of lævulose, and, moreover, is reduced to the same extent by dextrose and lævulose, thus simplifying the formulæ for calculating the compositions of mixtures. Polarisations are not involved. In working out the application of the method to various products Kruisheer has paid special attention to the interfering effect of fruits in jams, etc., owing to their unequal contents of dextrose and lævulose. The possible error in the estimation of glucose in jam due to this factor, is not more than 1 or 2 per cent. in nearly all cases, and is less than would be possible with other methods. A valuable feature of Kruisheer's system is that it enables a sharp discrimination to be made between commercial glucose and starch sugar. The method could, no doubt, be applied to more complex mixtures by bringing in the aid of the polarimeter.

A complication not well provided for in mixed sugar analyses is the presence of the type of dextrins occurring naturally as in honey, or formed by the action of acid on hexoses, as in the commercial preparation of invert sugar. Bruhns has described the latter class (*Z. angew. Chem.*, 1922, **35**, 61, 77; *J. Soc. Chem. Ind.*, 1924, *B*, 141), and has studied their behaviour with Fehling's solution and in the Clerget inversion, showing that they may be

determined by prolonged heating with hydrochloric acid. The dextrins of honey may be separated from the sugars, according to Lucius (*Z. Unters. Lebensm.*, 1926, **51**, 351 ; 1927, **53**, 376 ; *Analyst*, 1926, **51**, 581 ; 1927, **52**, 599), by precipitation with ether from an alcoholic solution. It is possible that a similar principle might prove of use in separating the dextrins of starch conversion products when present in mixtures.

DETERMINATIONS FOR PHYSIOLOGICAL PURPOSES

Determinations of sugar in physiological fluids have always had their special difficulties, leading to modifications of the usual processes. So long as these were confined mainly to urine analyses, the chief trouble lay in the difficulty of the end-point in Fehling's titrations : the familiar Pavy solution was designed to overcome this by retaining the cuprous oxide in solution by means of ammonia. The more convenient indirect volumetric copper methods have made possible a return to the ordinary Fehling's solution ; and for urine titrations a number of the newer methods are suitable (*e.g.*, Shaffer and Hartmann, Amick, etc.). The direct titration with methylene blue as indicator has also been successfully applied to urine by Lane and Eynon (*Analyst*, 1924, **49**, 366), either as a limit test or for exact determinations. Other methods now available are the Hagedorn and Jensen ferricyanide method (see below) ; and the picric acid colorimetric method, which has been specially adapted by Benedict and Osterberg (*J. Biol. Chem.*, 1921, **48**, 51 ; *Analyst*, 1921, **46**, 508) and by Duggan and Scott (*J. Biol. Chem.*, 1926, **67**, 287 ; *Brit. Chem. Abstr.*, 1926, *A*, 442). Certain disadvantages in the latter method for urine led Sumner to introduce 3-5-dinitrosalicylic acid as the basis of an improved reagent, which in its latest form is modified so as to eliminate the interference of certain non-sugar substances (*J. Biol. Chem.*, 1925, **65**, 393 ; *Analyst*, 1926, **51**, 45).

The great expansion in this field has, however, been due to the requirements of blood-sugar analysis, particularly since the discovery of insulin. The older process of Bang (*Thorpe's Dict. Appl. Chem.*, 1926, Vol. VI., 464) for titrating excess of copper

with hydroxylamine in presence of thiocyanate, was the most suitable method available for some time; but it required inconveniently large quantities of blood. A great step forward was made in 1919 by Folin and Wu, in devising a colorimetric method of determining directly very small amounts of cuprous oxide (*J. Biol. Chem.*, 1919, **38**, 81; 1920, **41**, 367; *Analyst*, 1920, **45**, 227). The precipitate was dissolved by a phosphomolybdic acid reagent to a blue solution, well suited for colorimetric purposes. To prevent back-oxidation of the cuprous oxide a special "sugar tube" was designed, which has since become standard equipment in biological laboratories. In the latest form of the process Folin employs an alkaline copper-tartrate reagent in which the alkalinity, due to a mixture of carbonate and bicarbonate, is quite weak; and a new acid molybdate reagent is also described (*J. Biol. Chem.*, 1926, **67**, 357; *Analyst*, 1926, **51**, 309). Minor improvements in this and the earlier method have recently been discussed by Folin (*J. Biol. Chem.*, 1929, **82**, 83).

The Folin-Wu reagents have been submitted to criticism by Benedict, who prefers to use a copper citrate reagent for the sugar, with a tungstic-arsenic-phosphoric acid reagent for developing a colour in a similar way to the phosphomolybdic acid (*J. Biol. Chem.*, 1926, **68**, 759; *Analyst*, 1926, 467). In a still more recent development, Benedict has modified his reagent so as to make it less subject to interference by non-sugar reducing substances in blood, by incorporating alanine, by replacing part of the citrate by nitrate, and by adding a certain amount of sulphite. With this it is possible to use the phosphomolybdic acid reagent of Folin and Wu, or a similar one, for the colour development (*J. Biol. Chem.*, 1928, **76**, 457; *Analyst*, 1928, **53**, 230). This latest Benedict reagent has itself been criticised by Everett (*J. Biol. Chem.*, 1929, **82**, 369; *Analyst*, 1929, **54**, 430.

The last word has not yet been said as to the respective merits of the Folin-Wu and Benedict methods. It seems possible that both give blood sugar values which are slightly high. For much routine work on blood, however, valuable relative results may be obtained by either method. A useful description of them is given by Yoe (*Photometric Chemical Analysis*, Vol. I.).

A phosphomolybdic acid reagent has been used volumetrically by Fontès and Thivolle for small amounts of reducing sugar of the order of a milligram (*Bull. Soc. Chim. Biol.*, 1927, **9**, 353; *Brit. Chem. Abstr.*, 1927, *A*, 690). They boil with Fehling's solution or a modified alkaline copper solution, remove the cuprous oxide by centrifuging or by filtration in a special apparatus, dissolve in the phosphomolybdic reagent, and titrate the blue liquid to a colourless end-point with permanganate.

This procedure, however, involves the separation of the cuprous oxide, which is always undesirable, owing to the risk of atmospheric oxidation, and because of traces of the oxide, held in colloidal form by protective non-sugar substances, passing through the filter (Pick, *Int. Sugar J.*, 1925, 50). To avoid this, Amick (*Chemist-Analyst*, 1928, **17**, 10; *Brit. Chem. Abstr.*, 1929, *A*, 459) adds dextrose solution and alkali separately to a boiling copper sulphate solution, and then treats the cooled mixture directly with phosphomolybdic reagent (as in the Folin-Wu methods). Titration with permanganate follows as before.

A special application of the Shaffer and Hartmann method to blood-sugar analyses has proved convenient and reliable within certain limits; it is perhaps less sensitive than some other methods for amounts of sugar below 25 mgrms. per 100 c.c. of blood. Somogyi found that the reduction is very sensitive to slight variations in *p*H, and in consequence modified the reagent and adapted the process for as little as 0·2 c.c. of blood (*J. Biol. Chem.*, 1926, **70**, 599; *Brit. Chem. Abstr.*, 1927, *A*, 69).

The ferricyanide reduction method, as already described, although designed primarily for physiological work, made little headway until Hagedorn and Jensen, in 1923, found a convenient iodometric procedure for determining the unreduced ferricyanide (*Biochem. Z.*, 1923, **135**, 46: **137**, 92; *J. Chem. Soc.*, 1923, **124**, ii., 265, 440). After heating the blood filtrate, etc., with the ferricyanide, they add a mixed reagent containing iodide and zinc sulphate. Interaction between the excess ferricyanide and the iodide takes place and the zinc removes the resulting ferrocyanide as it is formed, so that the reaction can proceed to completion. The liberated iodine is then titrated with thiosulphate, and the sugar

calculated from the excess of ferricyanide thus found, in an obvious manner. Filtrates from as little as 0·1 c.c. of blood are sufficient. A good account of the method is given in *Thorpe's Dictionary*, 1926, Vol. VI., 488.

Later workers have found the Hagedorn-Jensen method reliable, provided that the microchemical technique is satisfactorily followed; and it is now established practice in many laboratories (Martinson, *Biochem. Z.*, 1927, **185,** 400; *Brit. Chem. Abstr.*, 1927, *A*, 787). It has been applied to the determination of rather larger quantities of reducing sugars by Hanes (*Biochem. J.*, 1929, **23,** 99; *Analyst*, 1929, **54,** 349).

Other methods of using the ferricyanide reduction have been proposed, such as that of Van Slyke and Hawkins, depending upon the rate at which the ferricyanide solution is decolorised. This is simple and rapid, and has an accuracy of ± 5 per cent. of the sugar (*J. Biol. Chem.*, 1928, **79,** 739, 1929, **84,** 69, 79; *Brit. Chem. Abstr.*, 1928, *A*, 1358, 1929, *A*, 1477, 1478). Folin determines colorimetrically the ferrocyanide, formed by the reduction, as Prussian blue (*J. Biol. Chem.*, 1928, **77,** 421; 1929, **81,** 231; *Analyst*, 1928, **53,** 392; 1929, **54,** 246) and finds the results for blood sugar definitely lower (and presumably more correct) than by the Folin-Wu method.

The osazone formation has been used by Denigès in a micro-determination of dextrose. If the sugar amounts to not more than 4 mgrms. per c.c. the osazone remains entirely in solution, and can be determined colorimetrically (*Ann. Chim. Anal.*, 1923, II., **5,** 71; *Analyst*, 1923, **48,** 343). A biochemical principle which may have valuable developments is due to Somogyi (*J. Biol. Chem.*, 1927, **75,** 33; *Analyst*, 1927, **52,** 719), who found that dextrose can be quantitatively adsorbed and removed from solution by a suspension of baker's yeast. He applied the method for arriving at the true sugar content of blood. Many other sugars are not adsorbed at all, some only partly, so that the idea may prove useful in separating sugars from mixtures (Raymond and Blanco, *J. Biol. Chem.*, 1928, **79,** 649; *Analyst*, 1928, **53,** 669).

Comparative studies of the recent blood-sugar methods have

been made by Herbert and Green (*Biochem. J.*, 1929, **23**, 339) and Miyama (*Chem. Abstr.*, 1929, **23**, 3246).

This brief outline of current developments in regard to microbiological analysis is sufficient to show that the way of the analyst and biologist in choosing among numerous arbitrary procedures is going to be a hard one: and there is little to guide him in the field of experience with larger quantities of sugars. There is clearly a sharp divergence here from the older methods, overweighted as they have been by their concern with sucrose and its inversion products, and the gap must undoubtedly widen further.

CERTAIN ACCESSORY DETERMINATIONS

Many examinations of sugars or sugar products are incomplete without information as to the amount of certain foreign constituents. In this respect, water and ash content are of quite general interest. Developments in indirect methods for the former have already been touched upon in considering physical apparatus, and the current practice of twisting electrical conductivity determinations into indirect estimations of the ash has also been discussed. Beyond this, certain other aspects of each may be briefly considered.

Moisture.—Almost every laboratory has its special procedure for moisture determinations. Much work is continually being done, especially in America, on direct drying methods, but as yet nothing has been evolved capable of giving concordant results in different laboratories for a variety of products. The Spencer oven (*Int. Sugar J.*, 1921, 333, 701), which provides for the drawing of a large volume of heated air through the material to be dried, has shown that the drying can be considerably speeded up with a gain in concordance between analysts (no doubt simply owing to the standardisation it entails), but its results are none the less arbitrary. In regard to the use of ovens and desiccators, much that has been said in the Report of the Milk Products Sub-Committee of the Society of Public Analysts (*Analyst*, 1927, **52**, 102) is to the point.

There is probably more hope to be placed in refractometric or densimetric methods, when a proper study has been made of

the effects of sugars and other substances concerned. The general tendency with impure sugar products seems to be for the refractometer to give low results and the specific gravity high ones. According to Brewster (*J. Assoc. Off. Agric. Chem.*, 1924, **7**, 354; *Chem. Abstr.*, 1924, 2438) the mean between the two is often in agreement with the best results by drying.

A novel and simple physical method which may prove to have distinct advantages for sugar products depends upon the determination of the dielectric constant (Lampe, *Z. Spiritusind.*, 1929, **52**, 387; *Brit. Chem. Abstr.*, 1930, *B*, 165).

The direct determination of moisture by distilling with an immiscible liquid, such as toluene, has now come into wide use. It is rapid and avoids many weighings, and gives consistent results, though it cannot always be said whether they state the true moisture content. The limitations and conveniences of the method have been well surveyed by Jones and McLachlan (*Analyst*, 1927, **52**, 383). An improvement suggested by Rice (*Ind. Eng. Chem. Anal.*, 1929, **1**, 31; *Brit. Chem. Abstr.*, 1929, *B*, 298) is the addition of filter-cel, which permits the water to come off at a lower temperature.

Less success has attended the use of calcium carbide for direct estimations. It is usually sought to measure the acetylene evolved, but Bonwetsch has also made use of the rise in temperature occurring when sugar and carbide are mixed in definite proportions (*Z. Zuckerind. Czechoslov.*, 1922, **47**, 59; *Int. Sugar J.*, 1923, 161).

Ash. It has long been the custom in ashing raw sugars and sugar products to obtain the ash by sulphating, the liability to casual variations being so reduced. From the sulphated ash, one-tenth is deducted to reduce the figure to that of a carbonated ash. Evidence has now been piled up showing that the factor 0·9 is incorrect, a figure of about 0·8 being nearer to the truth (Mikolasek, *Z. Zuckerind. Czechoslov.*, 1922, **45**, 246; *Int. Sugar J.*, 1922, 216; Cook, *Facts about Sugar*, 1922, **15**, 418; *Int. Sugar J.*, 1923, 102). Some workers prefer to ash directly, without sulphating (Brewster, *J. Assoc. Off. Agric. Chem.*, 1923, **6**, 365; *Int. Sugar J.*, 1923, 329. Jamieson and Withrow, *J. Ind. Eng. Chem.*, 1923, **15**, 886; *Int.*

Sugar J., 1923, 435). Browne and Gamble emphasise the fact that ash results are largely conventional, in view of chlorine and sulphur losses (*Int. Sugar J.*, 1923, 436; 1924, 165).

Sulphur Dioxide.—Since the issue of the Preservatives Regulations, more attention has been given to traces of sulphur dioxide in commercial sugars. As a sorting test, the direct titration with iodine is satisfactory, according to Spengler and Brendel (*Z. Ver. deut. Zuckerind.*, 1927, 163; *Brit. Chem. Abstr.*, 1927, *B*, 686) and Jensen (*Analyst*, 1928, **53**, 133). A lead sulphide stain, obtained after reduction with zinc and acid as in the Gutzeit test, was proposed by Mann as a means of estimation (Ogilvie, *Int. Sugar J.*, 1926, 644); a very sensitive modification of this has been described by Bryan (*Analyst*, 1928, **53**, 589).

SUGAR FACTORY METHODS.

Analysis is a vital necessity in all control of sugar factory operations, but the scope of this survey does not permit of anything more than a mere reference to recent work on the improvement of factory analyses.

It is in this field that stereotyped methods are retained most tenaciously, on account of the large issues involved in changes. A recent paper by Honig, however, giving particulars of the latest methods used by the Java Sugar Experiment Station in the analysis of cane sugars, shows that in that area at least there is an attempt to keep abreast of new ideas (*Archief. Suikerind.*, 1928, **36**, 639; *Int. Sugar J.*, 1929, 266). A new procedure for reducing sugars is described, embodying Schoorl's titration; a determination of *p*H is specified; and colour is examined spectrophotometrically at various wave-lengths at a fixed *p*H.

In view of the growing importance of the beet sugar industry in this country, the recently published Czechoslovak "Uniform Methods" for beet factory analyses have special interest (*Int. Sugar J.*, 1929, 36). The determination of sugar in the beet itself is still a matter of some uncertainty as regards methods of extraction and allowance for the volume of the marc. The long-used Sachs-Le Docte process has been defended by one of its originators

in face of the later Krüger method (*Int. Sugar J.*, 1927, 214, 387, 488); and the alcohol extraction method has been criticised by Dolinek (*Z. Zuckerind. Czechoslov.*, 1927, **51**, 499; *Int. Sugar J.*, 1927, 614) on the grounds of overheating and the effect of basic lead acetate. Stanĕk and Vondrák find that the rotatory power of the invert sugar is affected by the basic lead in the hot digestion method, and if much is present, the usual manner of correcting the sucrose, as determined by direct polarisation, is inapplicable (*Z. Zuckerind. Czechoslov.*, 1927, **51**, 220; *Int. Sugar J.*, 1927, 503). More recent contributions to the question have been made by Dolinek (*Z. Zuckerind. Czechoslov.*, 1928, **52**, 329; *Chem. Abstr.*, 1929, 3120) and Saillard (*Planter and Sugar Mfr.*, 1929, **82**, 123; *Chem. Abstr.*, 1929, 3120).

The detection of minute traces of sucrose in condenser and wash waters, etc., is usually made by the α-naphthol test. Stevens (*J. Ind. Eng. Chem.*, 1923, **15**, 363; *Analyst*, 1923, **48**, 344) has shown that an equally delicate reaction is obtainable with cresol dissolved in a solution of Castile soap; and Matthews has made use of the blue colour formed by boiling with hydrochloric acid and molybdate (*Chemist-Analyst*, 1928, **17**, 8; *Analyst*, 1929, **54**, 43). A less sensitive test, but one to which only sucrose responds, is that of Kryz (*Oësterr. Chem. Z.*, 1921, **24**, 141; *J. Chem. Soc.*, 1922, **122**, ii., 233), depending upon colour reactions with ammonium nickel sulphate.

Much that has been said in the sections on polarimetry and reducing methods has special application in the analysis of factory products.

THE FUTURE

What is to be the trend of sugar analysis in the future? How will the laboratory be specially equipped for this branch of analytical work? The answers depend on how far ahead we care to look. In the near future it seems certain that there will be a displacement, perhaps a complete ousting, of gravimetric methods. The attraction and simplicity of the newer volumetric and colorimetric methods are bound to make great headway.

Concurrently, there will be an extension of the use of physical

methods. Modern conditions demand speedier results, and physical apparatus, whether by its own intrinsic measurements or as an auxiliary to chemical methods, can best meet this demand. Increasingly convenient apparatus will be developed for pH work, conductometric determinations, spectrophotometry, and electrotitrations. Electrical illumination for polarimeter and colorimeter, etc., will be followed by the perfecting of the photo-cell for reading such light-transmitting instruments. The ultra-violet lamp has already found its way into sugar work: perhaps the X-ray tube is not far behind.

On the other side of pure chemistry, an enlargement in the services of biology can be looked for, in adding to the accuracy of existing biochemical methods, and in providing selective direct determinations where at present only indirect methods are available.

All this means a large increase in equipment and in specialisation. The sugar laboratory will become chemical, physical, and biochemical—is already so, in fact—though different aspects will, of course, be emphasised according to particular requirements, whether for research, industry, food control, or otherwise. Specialisation within the laboratory will replace divergence of method outside it. The new equipment will be of necessity costly, and economic pressure will enforce for it some sort of standardisation. In the long run, this will reflect itself in methods as well as in apparatus: and if this, again, has the effect of sweeping away much of the litter of out-worn and parochial methods still scattered up and down the laboratories of the world, it will be all to the good.

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CHAPTER II

OILS AND FATS

By C. Ainsworth Mitchell, M.A., D.Sc., F.I.C.

International Critical Tables Relationship between Constants Separation of Glycerides Separation of Saturated from Unsaturated Fatty Acids Halogen Absorption of Oils and Fats Bromine and Iodine Methods Differential Absorption Identification of Iso-oleic Acid Classification of Oils by Iodine Values of Sterols The Thiocyanogen Value—Chemical Tests for Vitamins in Oils.

A SURVEY of the lines upon which processes for the analysis of oils and fats have been developed shows a great advance upon the accumulation of physical and chemical data with which the earlier chemists had, for the most part, to be content. The so-called constants of oils and fats are, however, still important factors for enabling a judgment to be formed as to the identity of an unknown specimen or the purity of a particular sample, and standard tables of figures for reference are a valuable analytical weapon.

Among the most recent and comprehensive of these tables are those compiled by Mitchell for *The International Critical Tables* (1927, Vol. II., pp. 196 to 217). In these tables 172 oils and fats are grouped in accordance with Alder Wright's scheme of classification, which is based partly on their origin and partly on their physical and chemical properties, and each oil or fat is given a general index number, by means of which it can be identified in the subsidiary tables. In these tables the individual oils are grouped by index numbers in ascending order of the value of the chemical or physical property, the intervals being indicated in bold type; thus the intervals for the saponification values are 50 units, and those for the iodine value 10 units.

The method of using the International Tables may best be shown by means of an example. An oil has been found on analysis to have the following properties: Solidification point, 0° C.; saponification value, 190; iodine value, 82; acetyl value, 11; unsaponifiable matter, 0·6 per cent.; melting point of fatty acids, 28° C.; "titre," 21° C.; n_D^{25}, 1·466. From each of the corresponding tables a list of the general index numbers lying in the neighbourhood of the experimental values is drawn up, and each list is arranged in the ascending order of these numbers. The number of times each index number occurs in this set of tests is ascertained. Thus in the particular example quoted, oil No. 8 occurred eight times; oil No. 31 seven times; oils Nos. 3, 5, 26, 47, 62 and 91, four times; oils Nos. 13, 20, 29, 33, 54, 61, 67, 83 and 84 three times. On turning to the general table (II.), and noting the properties there recorded for these oils, it will be found that all except No. 8 (olive oil) and No. 31 (neat's-foot oil) are eliminated. The oil must, therefore, be one of these or some oil resembling them, but not included in Table II. Further comparison of the values (*e.g.*, the acetyl and iodine values) eliminates No. 31, but additional confirmatory tests are, of course, required.

Relationship between the Constants.—It has been shown that there exists a definite relationship between certain of the physical and chemical constants of an ordinary unoxidised fat. Thus Backer (*Chem. Weekbl.*, 1916, **35**, 954) found the relationship between the refractive index n, the specific gravity d, and the saponification value V, and iodine value I, to be expressed by the equation:

$$\frac{n^2t - 1}{n^2t + 2} \times \frac{100}{d_4^t} = 33{\cdot}07 - 0{\cdot}00075\,\mathrm{I} - 0{\cdot}01375\,\mathrm{V} + 0{\cdot}002\,(t - 15).$$

When hydroxy acids are present the first figure of the equation is lower. Thus, with linseed oil the calculated figure was 30·56 and the observed figure 30·66, whereas with castor oil the calculated and observed figures at 15° C. were 30·63 and 29·51, respectively. Another equation, which takes into consideration the effect of the acid (A.V.) and saponification values on the refractive

index, is that worked out by Pickering and Cowlishaw (*J. Soc. Chem. Ind.*, 1922, **41**, 74T):

$$n_D^{40} = 1{\cdot}4643 - 0{\cdot}000046\,(\text{S.V.}) - 0{\cdot}0096\,(\text{A.V./S.V.}) + 0{\cdot}0001171 \text{ iodine value.}$$

The general conclusion was drawn that if a sample of oil shows a higher refractive index than that calculated from this equation, it may be inferred either that the oil was obtained from damaged material or that it is not fresh.

"Factors" derived from Constants.—A method of detecting and determining illipé fat in cocoa butter has been based by Tate and Pooley (*Analyst*, 1921, **46**, 229) on the use of "factors" obtained by multiplying together certain constants of the respective fats determined under standard conditions. The particular constants used for this purpose were the specific gravity at 60°/15·5° C., and at 90°/15·5° C., the viscosity at 60° C., the reciprocal of the iodine value, the melting point of the fatty acids, and the refractive index (n_D^{40}), and the average "factors," calculated by multiplying together the values for the respective pure fats, were: cocoa butter, 3,150; illipé fat, 4,103. When the method was applied to mixtures of known quantities of the two fats, results agreeing within 1·2 per cent. of the actual amounts were obtained. A correction for this error is found by reference to the following table, which gives the amounts to be added to the percentage of illipé fat determined:

Illipé fat observed, per cent.	0	10	20	30	40	50	60	70	80	90	100
Correction to be added	0·0	1·7	3·0	3·7	4·0	4·2	3·9	3·2	2·2	1·1	0·0

If only a small amount of fat is available a "short factor" is used, calculated from the melting point, melting point of fatty acids, and the reciprocal of the iodine value. These gave an average value of 4,166 for cocoa butter and 5,615 for illipé fat, with corrections ranging from 1·5 to 3·9 for amounts from 10 to 60 per cent. of illipé fat in a mixture, and then falling to 1·1 for proportions of 70 to 90 per cent.

Whymper and Bradley's criticism (*J. Soc. Chem. Ind.*, 1925, **44**, 9, 77) of this method is that it is essential that a standard

method of determining the melting points should be used, since existing methods may yield very varying results.

The principle developed to meet this particular instance should also be applicable to mixtures of other fats which closely resemble each other.

SEPARATION OF GLYCERIDES

Fractional Crystallisation. The earlier attempts of Heintz and of Hehner and Mitchell (*Analyst*, 1896, **31**, 329) to separate the individual glycerides in a fat by repeated crystallisations from ether have been continued with more success by the application of fractional crystallisation from more suitable solvents. Thus Seidenberg (*J. Ind. Eng. Chem.*, 1917, **9**, 855; 1918, **10**, 627) has devised a method in which the fat is dissolved in a mixture of two solvents, one of which is more volatile and dissolves more of the fat than the other, *e.g.*, alcohol and ether. Air is aspirated through the solution, with the result that evaporation of solvent and simultaneous chilling take place, and the glycerides which successively separate are removed, and subsequently added to similar deposits and subjected to further fractionation. In this way the mixed glycerides, oleodistearin and dioleopalmitin, were separated from beef fat, and the presence of beef or mutton fat in butter was indicated by the amount of insoluble glycerides obtained.

The physical properties of synthetically prepared triglycerides have been studied by Jaghker and Watson (*J. Soc. Chem. Ind.*, 1928, **47**, 365).

Methods of fractional crystallisation have also been applied by Bomer and Merten (*Z. Unters. Nahr. Genussm.*, 1922, **43**, 101) to isolate the various mixed glycerides in goose fat, and by Amberger and Wieschalm (*l.c.*, 1923, **46**, 276, 291) in studying the component glycerides of lard, successive fractionations being made from ether, from alcohol, and from acetone. They were found to include oleodistearin (m.p. 42° C.), oleopalmitostearin (m.p. 41° C.), and palmitodiolein (a colourless oil).

More recently Bomer and Ebach (*Z. Unters. Lebensm.*, 1928, **55**, 501) have separated the constituent glycerides from various oils and fats by a method of fractional distillation in a cathode

vacuum followed by fractional crystallisation of the distillates from acetone.

A new method, devised by van Raalte (*Rec. Trav. Chim. Pays-Bas*, 1929, **48**, 1058), consists in shaking 10 grms. of a solid fat with 10 c.c. of 96 per cent. alcohol and sufficient acetone (20 to 30 c.c.) to cause the crystalline portion to agglomerate, whilst the amorphous portion remains in solution and may be separated by filtration. The crystals are washed twice with 20 c.c. of a mixture of alcohol and acetone (1 : 1), dried and weighed, whilst the filtrate is evaporated and the residue dried and weighed. The iodine values and refractive indices of the different fractions may afford useful analytical information as to the nature of a fat.

Separation of Glycerides by Bromination. The mixed glycerides present in certain oils, notably some oils of the drying class and marine animal oils, readily yield an insoluble bromide on adding bromine to the oil dissolved in a suitable solvent. This behaviour, first discovered by Hehner and Mitchell (*Analyst*, 1898, **23**, 310), has long been used as an analytical method for the examination of linseed oil, and is not infrequently, though incorrectly, termed the "hexabromide" value. According to the original method, a weighed quantity of the oil is dissolved in ether, the solution chilled and treated drop by drop with bromine until a slight excess is present, the solution removed by means of a suction pump, and the insoluble bromide washed with chilled ether, dried and weighed. This method yields concordant results, provided that the conditions of working are kept exactly the same, but slight variations in the proportions of oil and solvent used or in the degree of washing will affect the amount of insoluble compound finally obtained. This is the reason why Gemmell (*Analyst*, 1914, **39**, 28, 388) was unable to get concordant values, and why the results obtained by Eibner and Muggenthaler (*Chem. Zentrbl.*, 1913, i., 567), after brominating the oils at a temperature of 10° C., were very much higher than those recorded by Hehner and Mitchell.

The explanation of the discrepancy lies in the fact that the insoluble bromides deposited in the original method are more or

less contaminated with other (more soluble) bromides, and that it is not easy to wash these completely out of the precipitate. In Eibner and Muggenthaler's modification the insoluble deposit formed was probably a mixture of bromides, and not mainly the most insoluble bromide. This was first established by Toms (*Analyst*, 1924, **49**, 77), who succeeded, in 1923, in finding a good solvent for the very insoluble bromide deposit from linseed oil, and, by recrystallising it from ethyl acetate, separated it into two mixed glycerides, namely, (1) a linolic-dilinolenic bromo-glyceride melting at 153° C.; and (2) trilinolic bromo-glyceride or an oleic-linolic-linolenic bromo-glyceride, melting at 117° C.

Toms's Method.—The method of determination used by Toms consisted in brominating 40 grms. of the oil dissolved in 300 c.c. of ether and 2 c.c. of glacial acetic acid, and adding 16 c.c. of bromine, slowly and with mechanical agitation, while the mixture was mechanically stirred for some hours. After standing overnight, the supernatant liquid was decanted, and the residue vigorously stirred with 100 c.c. of ether, this process being twice repeated, the mixture finally filtered, and the insoluble residue boiled for several hours with alcohol and dried on a porous tile. It was then dissolved in boiling ethyl acetate, and allowed to crystallise, the deposit boiled with alcohol, which was filtered off, and the final residue boiled with ether, filtered off, and left to dry on a porous tile. The 40 grms. of linseed oil yielded 18·7 per cent. of the pure crystalline bromide.

Eibner and Schmedinger (*Chem. Umschau*, 1923, **30**, 293) published a paper immediately after that of Toms's had been read, in which they isolated the same bromo-glyceride (*α*-dilinolenic-*α*-linolic bromo-glyceride) by dissolving the crude linseed oil bromide in hot tetralin (tetrahydro-naphthalene, $C_{10}H_{12}$), adding acetone until there were signs of precipitation, and then cooling the solution. The crystalline product melted at 156° C. This method of purification yields a somewhat purer product than that given by Toms's method.

Subsequently Toms (*Analyst*, 1926, **51**, 387) found that the insoluble bromides yielded by perilla oil, candlenut oil, and Para rubber-seed oil, when purified by solution and crystallisation

from tetralin and acetone, are identical in composition and melting point (156° C.) with the most insoluble bromide obtainable from linseed oil.

Oil Bromide Films.—The films left after bromination of a fatty oil and removal of the excess of bromine sometimes show distinctive characteristics, which may be of value for the rapid identification of the particular (Toms, *Analyst*, 1928, **53**, 72). Thus linseed oil and its distinctive fatty acid, linolenic acid, yield hard, colourless gritty films with a wrinkled surface, which is not very glossy, whilst boiled linseed oil gives a smooth, brown, glossy film. Candlenut oil, however, which has a high iodine value, gives a soft, sticky, glossy film, inclined to wrinkle, and perilla oil, again, gives a hard, wrinkled, dull film which tends to break up into streaks.

Tung oil gives an extremely glossy yellowish film, but soft and sticky and with no tendency to wrinkle, whilst menhaden oil yields an opaque, coarsely wrinkled, dull film. Castor oil films break up into transparent droplets, whilst apricot kernel oil gives opaque droplets. These differences appear to be due to the behaviour of the predominating glycerides in the respective oils.

Separation of Glycerides by Alcoholysis.—The method of separating glycerides by converting them into the methyl or ethyl esters of the fatty acids present, and separating these esters by fractional distillation, was termed "alcoholysis" by Haller (*Compt. rend.*, 1906, **173**, 657), and was used by him to ascertain the composition of coconut oil (*Id.*, 803). Elsdon's critical study of the method for investigating the composition of coconut oil and palm-kernel oil (*Analyst*, 1913, **38**, 8; 1924, **49**, 425) showed that it is too tedious for ordinary analysis, and that it does not yield quantitative results. A similar conclusion was drawn by Channon, Drummond and Golding (*Analyst*, 1924, **49**, 311) after studying the application of the method to butter fat, and they doubted whether results published by Holland and Buckley (*J. Agric. Proc.*, Washington, 1923, **24**, 365) and others were more than approximations to the real figures. Undoubtedly the process does afford information which cannot be readily obtained by other methods, but its quantitative limitations must be recognised.

Thus, Hilditch and Jones (*Analyst*, 1929, **54**, 76) have found

that, if a preliminary separation into groups of different character is effected, the method is capable of yielding results accurate to within about 1 per cent. In their experience, when analysing a fat containing a large number of glycerides in an ascending scale, such as butter fat, it is best to collect a series of small fractions over a range in which a particular ester is likely to be the chief component, since it is hardly practicable to isolate the individual esters, as suggested by Armstrong, Allan and Moore (*J. Soc. Chem. Ind.*, 1925, **44**, 631T). As a further precaution, each large primary fraction should be refractionated separately, and not added to the residue left on refractionating the preceding primary fraction; this renders the calculation of the results more exact.

For example, in analysing butter fat, the acids volatile with steam (butyric, caproic, caprylic and capric) are first distilled from the mixed fatty acids from 300 to 400 grms. of the fat, by a modification of the Reichert-Meissl process, continued for four to five hours, and the composition of these acids is determined by extracting them from the distillate with ether and fractionating them by distillation in the usual way.

The non-volatile fatty acids are then separated into solid and liquid acids by converting them into lead salts, which are treated with alcohol (see p. 62). Each group of acids is then converted into methyl esters, and these are fractionally distilled from a Willstatter bulb under a high vacuum. Where the resulting fractions show too wide a range in boiling point, they are refractionated. Finally, the fatty acids are recovered from the several fractions, and their saponification and iodine values are determined. The primary residues are saponified, unsaponifiable matter extracted with ether, and the fatty acids recovered and examined by the usual methods.

SEPARATION OF SATURATED FROM UNSATURATED FATTY ACIDS

The Lead Salt and Ether Method. The inadequacy of Varentrapp's original method, and of its modifications by Muter and others, has long been recognised (see Hehner, *Analyst*, 1892,

17, 181), and, although a partial separation or fractionation may be effected in this way, attempts have constantly been made to discover a more trustworthy process, and various other salts of the fatty acids have been tried, as well as other solvents.

Seidenberg (*J. Amer. Chem. Soc.*, 1921, **43**, 1323) found that, in the absence of highly unsaturated fatty acids, such as linolenic acid, a much more accurate separation may be effected by dissolving the lead salts in a mixture of three solvents—alcohol, chloroform and ether—two of which are more volatile than the third (alcohol), and then volatilising the solution by the aspiration of air until all the more insoluble salts are precipitated. The method was found to effect a quantitative separation of the lead salts of the saturated acids and to be independent of the proportions used.

Twitchell's Lead Salt and Alcohol Method.—In Twitchell's process (*J. Ind. Eng. Chem.*, 1921, **13**, 806), which has been widely adopted, the separation is based on the treatment of the lead salts with alcohol. The details of the method are as follows:

The fatty acids (about 2 grms. from a fat or 10 grms. from an oil) are dissolved in hot alcohol, and the solution treated with 1·5 grms. of lead acetate dissolved in boiling alcohol, the total alcohol used being 100 c.c. The flask is kept at 15° C. for eighteen hours, and the precipitate separated and washed on the filter with 95 per cent. alcohol, until the filtrate gives no turbidity with water. The precipitate is then boiled in a beaker with 100 c.c. of alcohol and 0·5 c.c. of acetic acid, the solution cooled as before, and the precipitate again collected and washed. Finally, it is decomposed with nitric acid and ether, the ethereal solution separated and washed with water and then evaporated, and the residue of fatty acids dried and weighed.

The iodine values of the solid fatty acids thus isolated were usually below 1, but the solid acids from hydrogenated cotton-seed oil gave a high iodine value, evidently owing to the separation of unsaturated solid iso-oleic acid with the saturated solid fatty acids.

Separation of Highly Unsaturated Fatty Acids.—A method of separating highly unsaturated fatty acids, such as clupanodonic acid and acids of the $C_nH_{2n-10}O_2$ series, from saturated and less unsaturated acids in marine animal oils has been based by

Tsujimoto (*J. Chem. Ind. Tokio*, 1920, **23**, 272; *Analyst*, 1921, **46**, 57) on the fact that the lithium salts of the former are soluble in acetone containing about 5 per cent. of water, whereas the lithium of the latter are insoluble.

About 5 grms. of the fatty acids are freed from unsaponifiable matter and dissolved in 20 c.c. of anhydrous acetone, and the solution neutralised with 4N-lithium hydroxide solution (phenolphthalein as indicator). The number of c.c. required is termed *a*. Then (6 *a*) c.c. of water and 75 c.c. of acetone are added to the solution, and the flask is corked and immersed in ice for two hours, after which the solution is filtered through a filter paper in a funnel surrounded by ice. When the temperature of the filtrate has risen to that of the room, 50 c.c. are evaporated, the residue of soluble lithium salts decomposed with dilute hydrochloric acid, the liberated fatty acids dissolved in ether, the solution dried with anhydrous sodium sulphate and evaporated, and the residue weighed.

This method, although it does not effect a complete separation, affords a valuable means of fractionation, and the small amounts of solid and less unsaturated fatty acids which will be present in the residue may be separated by conversion into methyl esters and by fractional distillation. Various fish oils thus examined by Tsujimoto yielded from about 15 to 30 per cent. of highly unsaturated fatty acids (with iodine values ranging from 203 to 360), whereas non-drying and semi-drying vegetable oils gave relatively small amounts (1·2 per cent. in sesame oil). Linseed oil, however, yielded 9·3 per cent., so that it would appear that the lithium salt of linolenic acid (or possibly of iso-linolenic acid) dissolves in the same way as the salts of the more highly unsaturated fatty acids of marine animal oils.

Bertram's Oxidation Method.—The method of separation devised by Bertram (*Chem. Weekbl.*, 1927, **24**, 226) has the advantage over previous methods, in that it determines all the unsaturated fatty acids, not merely those which are liquid at the ordinary temperature. The principle of the method is the conversion of the fatty acids into potassium soaps, which are then oxidised in alkaline solution by means of potassium permanganate; the saturated fatty acids are then extracted from the hydroxy acids formed in the oxidation, and purified by being twice con-

verted into their magnesium salts. The detailed steps of the process are as follows :—

Five grms. of the oil or fat are boiled for an hour under a reflux condenser with about 75 c.c. of approximately 0·5*N*-alcoholic potash, and the soap solution titrated with 0·5*N*-hydrochloric acid. After separation of unsaponifiable matter by extraction with petroleum spirit, the alcohol is expelled from the soap solution, 5 c.c. of potassium hydroxide solution (50 Bé.) added, and the mixture thoroughly cooled and treated, little by little and with constant shaking, with a solution of 35 grms. of potassium permanganate in 750 c.c. of water, care being taken to keep the temperature below 25° C. When oxidation is complete the excess of permanganate is removed by adding dilute sulphuric acid and sodium bisulphite solution. The solution is again extracted with petroleum spirit, the extract washed with water and filtered, the solvent distilled, and the residue dissolved in ammonia solution. The solution of ammonium salts is treated with 10 per cent. ammonium chloride solution and 15 per cent. magnesium sulphate solution, and the resulting precipitate of magnesium salts is warmed, filtered off after cooling, broken up in dilute sulphuric acid and reprecipitated. Sulphuric acid is again added, the liberated fatty acids extracted with petroleum spirit, the extract washed, filtered and distilled, and the residue weighed. The greatest error found in test experiments was − 0·7 per cent., and usually the error was much less.

Oxidation Method of Separation.—A new line of attack, which has recently been developed, has already proved its great value as a means of determining the nature of the glycerides in different fats. It was shown by Armstrong and Hilditch (*J. Soc. Chem. Ind.*, 1925, **44**, 43T) that, on treating an acetone solution of the methyl or ethyl esters of higher unsaturated fatty acids with powdered potassium permanganate, they were oxidised almost quantitatively into a monobasic fatty acid and the hydrogen ester of a dicarboxylic acid. Subsequently Hilditch and Lea (*J. Chem. Soc.*, 1927, 3106) extended the process to the natural glycerides of the fatty acids, which, when oxidised in acetone solution with solid permanganate, were found to yield nonoic, hexoic or propionic acid, together with complex glycerides combined with one or more azelaic acid residues.

Experimental oxidations showed in the case of natural fats the products given in the subjoined table (where *S* repre-

sents a saturated, and U an unsaturated fatty acid) might be formed :—

	Original Glyceride	Oxidation Product
Fully saturated	$C_3H_5(O.CO.S)_3$	$C_3H_5(O.CO.S)_3$
Mono-oleo derivative	$C_3H_5\begin{cases}(O.CO.U)_2\\(O.CO.S)\end{cases}$	$C_3H_5\begin{cases}(O.CO.S)_2\\(O.CO.[CH_2]_7.COOH\end{cases}$
Dioleo derivative	$C_3H_5\begin{cases}(O.CO.S)\\(O.CO.U)_2\end{cases}$	$C_3H_5\begin{cases}O.CO.S)\\O.CO.[CH_2]_7.COOH\end{cases}$
Triolein	$C_3H_5(O.CO.U)_3$	$C_3H_5(O.CO.[CH_2]_7.COOH$

The method as applied to an ordinary solid fat, such as mutton tallow, is as follows :

A solution of 100 grms. of the fat in a litre of anhydrous acetone is treated, little by little and with constant shaking, with powdered potassium permanganate, the flask being meanwhile kept beneath a reflux condenser, since the heat of oxidation causes the solvent to boil. The mixture is boiled for four hours beneath the reflux condenser, after which it is allowed to stand over-night. The acetone is then distilled, the residue mixed with water, and the excess of permanganate reduced by adding sodium bisulphite and the slightest possible excess of 30 per cent. sulphuric acid. The decolorised product is now extracted for several hours with successive large volumes of ether, the united ethereal extracts washed with water and evaporated to dryness, and the residue dissolved in hot alcohol and titrated with N alcoholic alkali. The neutral alcoholic solution is now poured into an aqueous solution of sodium carbonate, from which the neutral fat is removed by repeated extraction with ether.

In this way the acid products of the oxidation are finally separated from the neutral glycerides, which may then be further differentiated by fractional distillation after conversion into their methyl or ethyl esters (*cf.* p. 60).

The results thus obtained throw light upon the different physical properties of fats which contain approximately the same proportions of fatty acids. For example, Hilditch (*Chem. and Ind.*, 1929, **48**, 214) has shown that a cocoa butter which contained 58·8 per cent. of saturated and 41·2 per cent. of unsaturated glycerides yielded by this method only 2·5 per cent. of fully saturated glycerides, whereas a South American mutton tallow with 59·7 per cent. of saturated and 40·3 per cent. of unsaturated fatty acid, yielded 26 per cent. of fully saturated glycerides.

Hilditch further concludes that, as a rule, in seed fats the saturated fatty acids are distributed fairly uniformly among the glycerides, except that the proportion of oleic acid linked to saturated acids does not seem to exceed the ratio of 1 : 1·3 or 1·4, even in such fats as coconut or palm-kernel oils, in which the saturated fatty acids largely preponderate. The ratio for the cocoa butter mentioned above was 1·4, whilst the ratio for the mutton tallow was 0·9.

The fully saturated glycerides in this tallow were found to consist largely of palmito-distearins and dipalmito-stearins, with a small amount of tristearin, whilst the mixed saturated-unsaturated glycerides probably contained mono-oleo and di-oleo glycerides, with only a little triolein.

In the cocoa butter about 77 per cent. of the glycerides were mono-oleo di-saturated glycerides, largely composed of oleo-palmito-stearins. There was only a very small amount of fully saturated glycerides, and not more than 4 per cent. (on the whole fat) of tri-olein.

The method has also enabled a distinction to be drawn between the composition of pulp fats and seed fats. For example, whilst palm-kernel oil probably contains about 26 per cent. of mono-oleo-di-saturated glycerides, palm oil contains 37 per cent., or more, of those glycerides, together with some tripalmitin. Methods have still to be devised for the exact determination of the amounts of mono-oleo, di-oleo, or tri-oleo glycerides in mixtures of mixed glycerides containing one or more of them.

Distillation of Volatile Fatty Acids.—The method of determining the constituents in a mixture of volatile fatty acids by fractional distillation has become known, from the name of its originator, as the Duclaux process (*Ann. Chim. Phys.*, [5], ii., 223). During the period under review, there have been several investigations dealing with the principles of the process. Gillespie and Walters (*J. Amer. Chem. Soc.*, 1917, **39**, 2027) showed that the method was applicable to analysis, and gave formulæ for calculating the results. Some of their results were afterwards criticised by Richmond (*Analyst*, 1919, **44**, 255), on the ground that they had not used pure specimens of the respective acids for establishing their experimental values.

For this reason Richmond redetermined the rates of distillation for the various pure acids, and embodied his results in a series of tables giving the mean of a number of distillations of acid of various strengths.

In view of the discrepancies introduced by the effects of condensation, an apparatus previously devised (*Analyst*, 1908, **33**, 305) was used, in which condensation is, to a large extent, eliminated. This comprised a 300 c.c. flask, the outlet tube of which was bent over and connected with a vertical Liebig's condenser. The flask rested on asbestos board in which a circular hole had been cut, and both flask and outlet tube were surrounded by a cylindrical vessel filled with water. This was boiled over a small flame, and fractions of about 10 c.c. each were collected from 100 c.c. of the acid solution in the flask, each fraction being measured to the nearest 0·05 c.c. To eliminate the effect of any carbon dioxide, a blank determination was made by distilling 100 c.c. of distilled water, and the results obtained were deducted from the actual titration figures. An apparatus of this or similar type should be used with the tables for the acids.

The tables also include a column $\frac{\Delta y}{\Delta x}$, giving the percentage to be added to, or subtracted from, the quantity stated for each 1 per cent. of volume above or below that quantity, up to a maximum of about 0·5 per cent.

HALOGEN ABSORPTION OF OILS AND FATS

From the time when von Hubl, in 1884, devised his method of determining the halogen absorption of oils and fats, the iodine value has been one of the most important factors in the analysis of oils and fats, and the publication of new processes and modifications of the original method has continued in a never ceasing flow. The original Hubl method, however, is but little used at the present day, since the Wijs method and the Hanus method have been found much more rapid and more widely applicable, and still more recently the supremacy of these has,

in turn, been challenged by the pyridine sulphate bromide method and the alcoholic iodine method of Margosches.

In view of the necessity of having a method which would yield concordant results in the hands of different workers, the conditions for using both the Hanus and the Wijs methods have been standardised.

The Hanus Method as a Standard. A German committee was appointed to study the question of the iodine absorption of fats, and a report on the subject was issued by Auerbach in June, 1926 (*Chem. Umschau*, 1926, **33**, 187). According to Auerbach, the Hanus method had been almost unanimously approved as the best standard method, whilst the Margosches method had not given satisfactory results with tung oil and certain marine animal oils, and Kaufmann's bromine solution method still required further experimental study.

Standard Wijs Method. At the Ninth Conference of the International Union of Pure and Applied Chemistry at The Hague, in 1928, the question of the standardisation of the iodine value was discussed, and a resolution was adopted that the Wijs method should be given the preference, especially in forensic work, and that it should be accepted as the official method.

Wijs (*Analyst*, 1929, **54**, 12) has defended his method from certain criticisms which had, in the past, been brought against it, and has shown that some of these were based on the use of a reagent of incorrect composition. The general directions for using the method, as standardised in the Report of the Conference, are as follows :

> "The best method of preparing the reagent is to dissolve about 9 grms. of iodine trichloride in 1,000 c.c. of glacial acetic acid of at least 99 per cent. strength. If the liquid can be kept in a cool place where crystallisation can take place, a mixture of 300 c.c. of carbon tetrachloride and 700 c.c. of acetic acid may be used instead of 100 c.c. of acetic acid.
>
> "Exactly 5 c.c. of this solution are taken, and its halogen content determined by means of N/10 thiosulphate solution, after addition of potassium iodide and water. The bulk of the solution is treated with 10 grms. of pulverised iodine and shaken to make it dissolve. When almost all the iodine has dissolved, exactly 5 c.c.

are again taken and the halogen content determined. As soon as this is found to be one-half more than that found in the first determination, the solution is filtered into a bottle provided with a tightly fitting stopper. It is preferable to exceed slightly this limit of one-half more, as this ensures that no iodine trichloride remains in the finished reagent: this would make the preparation unstable. If desired, the reagent may be diluted with acetic acid to exactly *N* 5 strength.

"The acetic acid and carbon tetrachloride must be absolutely free from oxidisable matter; this is controlled by warming 1 or 2 c.c. of each liquid with a little strong sulphuric acid and a drop or two of a saturated solution of potassium dichromate. A green tinge should not be noticeable, even after prolonged standing. Kept in a well-closed bottle in the dark, the solution remains in good condition for years.

"It must be borne in mind that the coefficient of expansion by heat is rather high (0·00115); thus for 25 c.c. a difference in temperature of 1° C. makes a difference in the titration with *N* 10 thiosulphate of 0·06 c.c.

"The quantity of oil or fat taken for a test should be so measured that not more than 30 per cent. of the halogen present in the 25 c.c. of the solution added is absorbed. This quantity of oil is dissolved in a few c.c. of carbon tetrachloride before the 25 c.c. of the reagent are added.

"After 30 minutes to 2 hours, according to the degree of unsaturation of the fat or oil, the non-absorbed halogen is titrated."

Pyridine Sulphate Bromide Method.—A new method of determining the halogen addition of an oil without risk of substitution or oxidation has been introduced by Rosenmund and Kuhnhenn (*Z. Unters. Nahr. Genussm.*, 1923, **46**, 154). The reagent is prepared by dissolving 8 grms. of pyridine and 10 grms. of sulphuric acid in 20 c.c. of chilled glacial acetic acid, adding a solution of 8 grms. of bromine in 20 c.c. of acetic acid, and diluting the mixture to 1 litre with glacial acetic acid.

To determine the iodine value, the weighed quantity of the oil or fat is dissolved in 10 c.c. of chloroform, the solution treated with a slight excess of the reagent, and allowed to stand for three to five minutes. The excess of pyridine sulphate bromide is then determined by adding potassium iodide solution and titrating the liberated iodine, or the excess may be ascertained by direct titration with standard arsenite solution.

The iodine value thus rapidly determined agrees with that found by the Wijs method, and it has the advantage that it is not dependent on the addition of a large excess of the reagent. The solution keeps well, but requires restandardising from time to time.

Margosches' Alcoholic Iodine Method. The principle underlying the rapid method of determining the iodine absorption of oils devised by Margosches, Hinner and Friedmann (*Z. angew. Chem.*, 1924, **37**, 334) is that an alcoholic solution of iodine reacts differently towards fats in the presence of water from when it is used alone. So far back as 1884 Hübl (*Dingl. polyt. J.*, 1884, **253**, 281) showed that alcoholic iodine by itself is absorbed only slowly and irregularly, but if an emulsion is formed of the iodine solution, water, and the fatty oil, hypoiodous acid and hydriodic acid are formed, and the sum of the two gives the iodine absorption.

From 0·1 to 0·15 grm. of the oil (according to the unsaturation) is dissolved in 10 c.c. of absolute (99·8 per cent.) alcohol at the ordinary temperature, or in 15 c.c. of 96 per cent. alcohol at about 50° C., in a 500 c.c. flask on the water bath, and the solution cooled and treated with 20 c.c. of *N*/5 alcoholic iodine solution (2·54 grms. of iodine in 100 c.c. of 96 per cent. alcohol). The mixture is shaken, diluted with water, allowed to stand for five minutes (not longer), and the excess of iodine titrated with *N* 10 thiosulphate solution. Simultaneously a blank test is made and the correction thus obtained deducted from the result.

The method is modified as follows for solid fats :

From 0·2 to 0·4 grm. of the fat is dissolved in 10 c.c. of absolute (99·8 per cent.) alcohol on the water-bath, and the hot solution cooled to about 25° to 30° C. (not to the ordinary temperature, as in the case of oils), so that the liquid still remains clear. It is then treated with 20 c.c. of *N* 5 alcoholic iodine solution (or with 10 c.c. only if the iodine value of the fat is very low), the mixture shaken and diluted with 200 c.c. of water (at about 20° C.) which produces an emulsion, after which the procedure used for fatty oils is followed.

To obtain results in agreement with those given by the Hanus method, it is essential that there should be an excess of 70 per cent. of iodine. The immediate addition of potassium iodide, as in the ordinary Hübl method, must be omitted, or the addition of the hypoiodous acid to the fat will not proceed regularly.

Holde (*Chem. Umschau*, 1925, **32**, 147) made a series of comparative tests with the method, and found that it gave results in close agreement with those obtained by the Hanus method. Both methods, however, failed with cholesterol, though Margosches (*Die Jodzahlschnellmethode*, p. 95) states that, with suitable modifications, his method is also applicable to sterols.

Tung oil (Japan wood oil) behaves abnormally with the Margosches reagent. In respect of this oil, the methods of Wijs and of Hanus also fail. The explanation of this has been found by Boeseken and Ravenswaay (*Rec. Trav. Chim. Pays-Bas*, 1925, **44**, 241) to be due to the presence of three unsaturated double bonds in α- and β-eleostearic acid (the characteristic acids of tung oil). Two of these bonds are saturated fairly rapidly, but the third requires a very long time for its saturation by the ordinary liquid reagents used for determining the iodine value (*cf.*, however, bromine vapour method).

The Super-iodine Value.—It has been shown by Margosches, Friedmann and Tschörner (*Ber.*, 1925, **58**, 794, 1064) and by Margosches and Fuchs (*Ber.*, 1926, **59**, 375) that by prolonging the absorption in the alcoholic iodine method to twenty-four hours, values are obtained which differ with oils having similar iodine values, and thus enable them to be distinguished.

The reactions involved are as follows :

I. The iodine of the alcoholic iodine solution reacts with the water present, to form hypoiodous acid—

$$I_2 + HOH \rightleftarrows IOH.$$

In the presence of a fat this system of equilibrium is pushed towards the right, and the sum of the hypoiodous and hydriodic acids formed gives the iodine value.

By continuing the action of the iodine beyond the five minutes, the iodine absorption increases, and, parallel with it, there is an increase in the amount of hydriodic acid produced, which in the five minutes' period accounts for about 50 per cent. of the iodine absorption.

The second reaction involved in the process is that indicated in the following equation, which represents the action of part of

the hypoiodous acid produced on fats or fatty acids containing unsaturated bonds—

$$R_1 . CH : CH . R_2 + IOH \rightarrow R_1 . CHI . CH(OH) . R_2.$$

A further quantity of hydriodic acid is also produced from the hypoiodous acid.

The super-iodine value is therefore defined as the measure of twice the quantity of hypoiodous acid (expressed in percentage of iodine) which is consumed in twenty-four hours in the action of alcoholic iodine solution on oils and fats. It depends to a large extent upon the excess of iodine used, and this must not fall below 70 per cent. excess.

Isomeric fatty acids behave quite differently in their reaction over the twenty-four hours' period. For example, ordinary oleic gave an iodine value of 107 after twenty-four hours, whereas the iodine value calculated from the amount of hydriodic acid was the normal figure of 84·5. Elaidic acid, on the other hand, gave the same value as oleic acid after the five minutes' absorption, whereas after twenty-four hours it gave an iodine value of 92·3; but the value calculated from the hydriodic acid was only 74·2.

Castor oil and olive oil, which have approximately the same iodine values, as was also shown by the absorptions after five minutes, gave quite different super-iodine values, castor oil giving 162·2 and olive oil 119·7.

The method appears to contain possibilities of further development, but at present seems to rest upon an empirical foundation, and to depend upon the exact observance of the specified conditions.

Bromine Vapour Method. Another rapid method of determining the halogen absorption of minute quantities of oils and fats is that devised by Toms (*Analyst*, 1928, **53**, 71). A single drop of the oil (from 0·02 to 0·03 grm.) is spread in the finest possible film on a weighed microscope slide, weighed, and exposed to bromine vapour in a wide tube closed at each end with a waxed cork. After twenty to thirty minutes the slide is either heated at a temperature not exceeding 50° to 60°, or is exposed to a current of warm air to remove the excess of bromine, and is again weighed.

The increase in weight, due to the absorption of bromine, is then calculated into the iodine value.

Compared with the Wijs method as the standard, this method gives very similar results for most unoxidised or non-hydroxylated oils and fats, including drying oils, semi-drying oils, non-drying oils, fish and marine animal oils.

It is essential, however, that only minute quantities of the oil should be used, since if the oil film is too thick, a long time is required to expel the excess of bromine, with a consequent risk of substitution taking place. In a method based on the same principle, Sabalitschka and Dietrich (*Pharm. Ztg.*, 1924, **69**, 425) used quantities of 0·1 to 0·4 grm. of the oil, and heated the plates for periods of two to three hours, so that it is not surprising that they observed some substitution.

This was also the drawback of the gravimetric bromine method devised by Hehner (*Analyst*, 1895, **20**, 49). Under strictly empirical conditions, the method gave fairly good results, but as the bromine and oil were mixed together in a solution, and a considerable period of heating was required to expel all the excess of bromine, some substitution was liable to occur and the weight of the oil bromide seldom became quite constant.

One advantage of the bromine vapour method is that the addition of halogen takes place much more rapidly and completely than when the Wijs or Hanus reagent is used. Thus with tung oil, which contains a large proportion of α eleostearic acid, the usual time of absorption (two hours) allowed with the Wijs reagent is insufficient for complete saturation. It has been shown by Boeseken (*Rec. Trav. Chim. Pays-Bas*, 1927, **46**, 619) that the addition of iodine monochloride takes place in three stages, the first of which is complete in a few minutes and the second in two hours, whereas the third is not complete until after six days. Hence, the iodine values previously recorded for tung oil represent the two-thirds absorption.

Bromine vapour, however, acts quite differently from iodine monochloride, and saturates simultaneously all three of the unsaturated double bonds of eleostearic acid—

$$C_4H_9.CH{=}CH.CH{=}CH.CH{=}CH(CH_2)_7.COOH.$$

Determination of *a*-Elæostearic Triglyceride in Tung Oil. This behaviour of bromine vapour has been used by Toms (*Analyst*, 1928, **53**, 75) as the basis of a method of determining the amount of elæostearic triglyceride in tung oil. The iodine value is first determined by the Wijs method, to obtain the normal value for non-conjugated systems of double bonds (A); then the iodine value is determined by the bromine vapour method, to obtain the complete halogen saturation of the non-conjugated double bonds and also of the *a*-elæostearic glyceride (B), the molecular weight of which is 872 (theoretical iodine absorption for 12 atoms of iodine = 174·8, and for 18 atoms of iodine = 262·5).

If, with a given oil, the mixture (A + B) absorbs p per cent. of iodine in two hours by the Wijs method, and q per cent. by the bromine vapour method, then

$$\text{A} \quad 262{\cdot}5 - 174{\cdot}8 - (q - p)$$

$$\text{B} \quad q - p$$

$$87{\cdot}7 \quad (q \quad p)$$

$$q \quad p$$

whence, for any sample the percentage of

$$\text{B} \quad \frac{(q - p)100}{87{\cdot}7}.$$

Applying this method to six different specimens of tung oil, Toms found them to contain the following proportions of elæostearic triglyceride: 64·09, 63·29, 71·7, 68·5, 72·8, 55·8, and 74·8 per cent.

Chlorine Vapour Method. Croxford (*Analyst*, 1969, **54**, 145) has made a comparative study of the bromine vapour method, in comparison with the Wijs method, a bromine solution method, and with a chlorine vapour gravimetric method. It was found that with ordinary unoxidised oils (linseed, soya bean, maize, olive, almond, whale) fairly concordant results were obtained by the first three methods, whereas the chlorine vapour method sometimes gave too high results, owing to substitution taking place. This substitution, however, reached a maximum.

Castor oil and ricinoleic acid gave considerably higher results by

the bromine vapour method than by the Wijs method, and still higher values by the chlorine vapour method. In each case, however, the values attained a constant figure, and so the method may eventually prove of analytical value. The substitution in the chlorine vapour method was very pronounced with arachis oil, reaching a maximum of about 160 to 170 in seven hours with different specimens of the oil.

Differential Halogen Absorption as Evidence of Constitution. It was shown by Ponzio and Gastaldi (*Gazz. Chim. Ital.*, 1912, **42**, 92) that when the double bond in an unsaturated fatty acid of the acrylic series occurs next to the carboxyl group, absorption of halogen from Hubl's, Wijs' or Hanus' solutions is abnormally slow, although by prolonging the absorption for several days saturation does eventually take place. This accounts for the anomalous behaviour of croton oil, the predominant acid of which is tiglic acid, $CH_3CH=C(CH_3)COOH$. The iodine value of this oil is usually given at about 108 to 109, but by prolonging the absorption with Wijs' solution and taking care that a large excess of halogen is present, iodine values of about 117 are ultimately obtained (Margosches, *Die Ludbls Jodmethode*, 1927, pp. 12, 20).

Toms showed (*vide supra*) that his gravimetric bromine vapour method gives complete absorption with tung oil, which contains a fatty acid with three double bonds, and more recently Croxford has found that the bromine vapour method effects rapid saturation of the fatty acids of the oleic series (and their glycerides) which give anomalous results with the Wijs reagent. For example, crotonic acid, $CH_3CH=CHCOOH$, with a theoretical iodine value of 295·4, gave an iodine value of 86·7 after eighteen hours' contact with Wijs' solution, rising to 190·3 after fourteen days, whilst the iodine value calculated from the bromine vapour absorbed in one hour was 296·2. In like manner, tiglic acid gave an iodine value of 96·6 in three days (Wijs), as compared with 249·7 (bromine vapour method); (theory = 254).

Croton oil gave 109·1 after two hours (Wijs), rising to 123·4 after fourteen days, whilst by the bromine vapour method it gave 112·4 after one hour, and 118·3 after seven hours.

Parsley-seed fatty acids, consisting largely of petroselinic acid

(*i.e.*, isomeric 6·7-oleic acid), behaved like the normal (9·10) oleic acid by both methods, as was to be expected.

It would thus seem feasible to ascertain the constitution of a fatty acid of the oleic series by differential absorptions for two hours by means of the Wijs reagent and by the bromine vapour method. If the results show approximate agreement, it is probable that the double bond of the fatty acid is not in juxtaposition to the carboxyl group, but, if there is a pronounced difference between the two results, the fatty acid is probably one having its double bond adjoining the carboxyl group, as is the case with crotonic acid, 2·3-hypogæic acid, and 2·3-oleic acid, $CH_3(CH_2)_{14}$=CH:CH.COOH.

Identification of Iso-oleic Acid

Williams and Bolton (*Analyst*, 1924, **49**, 460) have based a method of detecting iso-oleic acid in fats on the fact that its lead salt is nearly insoluble in many organic solvents, so that it behaves like the lead salts of saturated fatty acids when treated with alcohol as in Twitchell's method (*cf.* p. 62).

As iso-oleic acids, notably the 11-12-acid, are formed in the hydrogenation of oils, the detection of an isomeric oleic acid in a fat affords presumptive evidence that it has been hardened by a hydrogenation process. For this purpose the iodine value of the solid fatty acids, thus separated, is determined, and if it does not exceed 5 units (this allowance being made for incomplete separation of the liquid fatty acids), the absence of hydrogenated oil in the fat may be inferred.

The percentage of iso-oleic acid is obtained by the equation

$$i = \frac{95}{100} \cdot S \cdot \frac{I - 5}{90},$$

where S represents the percentage of solid fraction, and I its iodine value.

Iodine Value of Sterols of Fats

Bolton and Williams (*Analyst*, 1930, **55**, 1) have introduced a valuable new process into the analysis of fats by separating the

unsaponifiable matter under standard conditions, and determining its iodine value by the pyridine sulphate bromide method (p. 69). It was found that the Wijs method gave discordant results, in which respect the work confirmed the experience of Margosches.

The Bolton and Williams Method. "From 2 to 2·5 grms. of the oil are saponified by boiling under a reflux condenser with 25 c.c. *N* 2 alcoholic potash. The solution is titrated with *N* 2 HCl, using phenolphthalein as indicator. Five c.c. of *N* 2 NaOH are added, and the solution is extracted three times (or more in the case of certain fish oils) with petroleum spirit. The extracts are combined, washed with water, and filtered into a weighed flask, the spirit is evaporated off, and the contents dried and weighed. In general, this procedure will yield a quantity varying between 0·01 and 0·04 grm. of unsaponifiable matter free from soap and fatty acids.

"The unsaponifiable matter is dissolved in 5 c.c. of chloroform, and a quantity of the pyridine sulphate bromide reagent sufficient to leave an excess of unabsorbed halogen approximately equal to the amount absorbed is added; usually this will be 10 c.c. of the reagent. The mixture is allowed to stand in the dark for five minutes; 5 c.c. of 10 per cent. potassium iodide solution are added, and the iodine liberated is titrated with *N* 20 sodium thiosulphate solution. A blank experiment is carried out in the same manner with the reagents. The amount of halogen absorbed is calculated in terms of iodine as a percentage of the weight of unsaponifiable matter taken. It is essential, in conducting this test, that the method prescribed for obtaining the unsaponifiable matter be strictly adhered to, in order that this substance may be prepared in the necessary state of purity.

"Duplicate determinations, on a given sample, give results that do not vary by more than about 3 per cent. of the observed figure at the most."

Classification of Oils by the Test.—It was found by Bolton and Williams that, with the exception of soya bean oil, all the oils of common occurrence examined give an iodine value which falls within one of four narrow limits.

The natural oils can thus be classified into four groups, as shown in the following table :

IODINE VALUE OF THE UNSAPONIFIABLE MATTER OF OILS

Group 1. Iodine value 64 to 70.		*Group* 2. Iodine value 90 to 96.		*Group* 3. Iodine value 117 to 124.	*Group* 4. Iodine value 197 to 205.
Animal oils and fats.	Vegetable oils.	Fish and marine animal oils.	Vegetable oils.	Vegetable oils and fats.	Vegetable oil.
Beef	Kernel oils of *Palmae* :	Cod-liver	Cocoa butter	Almond	Olive
Butter-fat	Coconut	Herring		Arachis	
Ghee	Palm-kernel	Seal		Borneo tallow	
Lard	Babassu	Sardine		*Cotton	
		Whale		Grape-seed	
				Linseed	
				Maize	
				Palm	
				Rape	
				Rubber-seed	
				Sesame	
				†Soya	
				Sunflower	
				Tea-seed	
				Tung	

* Containing less than 2 per cent. unsaponifiable matter.
† After refining with alkali and bleaching with fuller's earth and charcoal.

The great value of this new method lies in the fact that it separates olive oil into a class by itself, and, for the first time, enables a chemical test to be applied for the detection of the substitution of tea-seed oil for olive oil.

It is probable that the use of differential adsorption methods applied to the sterols will enable the natural oils to be sub-divided into further distinctive groups.

The Thiocyanogen Value

A new principle has been utilised by Kaufmann (*Chem. Ztg.*, 1925, **49**, 768) in his method of determining the amount of free thiocyanogen absorbed by an oil or fat, since the value thus obtained does not always coincide with the iodine value, for there may be partial or complete absorption, or none at all. For example, with linolic acid and its glyceride, the thiocyanogen radicle is absorbed only at one double bond. Thus, by determining both the iodine value and thiocyanogen value of an oil, it is possible to calculate the amounts of the respective un-

saturated constituents. The process is as follows (*Arch. Pharm.*, 1925, 1):

The best solvent is anhydrous glacial acetic acid (obtained by distilling 99 per cent. acid over phosphoric anhydride, and collecting only the fraction distilling at 118° to 120° in a receiver with a calcium chloride seal to prevent absorption of moisture). The glacial acetic acid is divided into two portions, to one of which sufficient bromine to give a solution of the required normality (plus 5 per cent. excess) is added. In the second flask is suspended the quantity of pure lead thiocyanate (previously dried over phosphoric anhydride) to react with the bromine in the other flask (plus 50 per cent. excess). $Pb(SCN)_2 + Br_2 = PbBr_2 + (SCN)_2$.

The bromine solution is added, with continual shaking, to the contents of the second flask, and the mixing continued until decolorisation is complete, after which the solution of free thiocyanogen is filtered through a dry filter. It is standardised by adding a measured volume of it to potassium iodide solution, and titrating the liberated iodine with thiosulphate solution. To prevent polymerisation, the reagent should be kept in the dark and standardised before use.

Thiocyanogen Value of Fatty Oils. From 0·1 to 0·2 grm. of the oil is dissolved in sufficient 0·1*N* or 0·05*N* solution of the reagent to give an excess of 100 to 500 per cent. of thiocyanogen. After standing for five to fifteen hours (according to the character of the oil) the mixture is poured into potassium iodide solution, and the liberated iodine titrated. For comparison, the results are expressed as iodine values.

An alternative and simplified method of preparing the reagent, which utilises the method of dehydrating glacial acetic acid by means of acetic anhydride described by Gerber (*Seifensied. Ztg.*, 1928, **55**, 27), is as follows (Kaufmann, *Seifensied. Ztg.*, 1928, No. 35):

Glacial acetic acid (99 to 100 per cent.) is mixed with 10 per cent. of acetic anhydride and allowed to stand for some time. About 5 grms. of lead thiocyanate are then put into a flask with a closely fitting stopper, and the flask is filled up with the mixture and left for as long a time as can be spared. Then, before use, about 0·6 c.c. of bromine is run into the flask, which is thoroughly shaken until the liquid is colourless, after which it is filtered. With the reagent thus prepared the end-point of the thiocyanogen absorption is sharply indicated, even with highly unsaturated oils.

Oleic, elaidic, erucic and brassidic acids were found to give

values agreeing with the iodine values (Hanus); that is, the double bonds of these acids and their glycerides are quantitatively saturated with thiocyanogen. Fatty acids containing a triple bond, however, such as stearolic and behenolic acids, show no absorption at all, and linolic acid absorbs only half of the thiocyanogen corresponding to its iodine value (viz., 82·5 instead of 169·1); hence linolic acid absorbs thiocyanogen at only one double bond.

Indirect Determination of Linolic Acid. The method is illustrated by an example. The iodine value of a mixture of linolic, oleic and palmitic acids was 110·36, whilst the iodine value, calculated from the thiocyanogen value, was 62·37. Then, if x represents linolic acid (iodine value 181·09; iodine value from thiocyanogen = 90·54), y, oleic acid (iodine value 89·9), and z the saturated palmitic acid,

$$\text{I. } x + y + z = 100$$
$$\text{II. } 1{\cdot}810x + 0{\cdot}899y + 0z = 110{\cdot}36$$
$$\text{III. } 0{\cdot}9504x + 0{\cdot}899y + 0z = 62{\cdot}37$$

whence $x = 53$ per cent.; $y = 16$ per cent.; and $z = 31$ per cent.

By applying this method to a number of oils, the following amounts of linolic acid were found:

Oil.	Per cent.
Castor . . .	1·5
Olive . . .	4·9
Arachis (1) . .	22·45
,, (2) . .	23·14
Almond (1) . .	16·0
,, (2) . .	14·8
Rape . . .	33·1
Sesame . . .	36·8

In further work on the subject (*Z. Unters. Lebensm.*, 1926, **51**, 15) Kaufmann found that the iodine and thiocyanogen values of cocoa butter agreed, and that the fat was therefore free from linolic acid, whilst poppy-seed oil gave values corresponding with 27·5 per cent. of triolein and 63·8 per cent. of trilinolin. Five

specimens of arachis oil gave values corresponding with the following proportions of oleic and linolic acids :

	I.	II.	III.	IV.	V.
Oleic acid, per cent. . .	80·7	58·6	55·7	63·9	61·3
Linolic acid, per cent. .	10·0	22·4	23·1	18·6	19·7

Analysis of Mixtures.—In many cases it is possible to calculate the proportions of fats in a mixture from the results obtained by the two methods. For example, a mixture was made of poppy-seed oil (with iodine value 133·6, and iodine value from thiocyanogen absorption 78·7), arachis oil (iodine value 88·0 and thiocyanogen iodine value 68·1) and coconut oil (iodine value by each method, 9·4). The mixture had an iodine value of 74·19 and a thiocyanogen iodine value of 51·94.

By the use of the following equations the respective proportions were calculated :

$$x + y + z = 100$$
$$133{\cdot}6x + 88y + 9{\cdot}4z = 74{\cdot}19 \,.\, 100$$
$$78{\cdot}7x + 68{\cdot}1y + 9{\cdot}4z = 57{\cdot}94 \,.\, 100$$

and the following percentages were found :

	Taken.	Found.
Poppy-seed oil . .	25·06	24·51
Arachis oil . .	42·96	43·06
Coconut oil . .	31·98	32·48

Obviously the presence of any considerable amount of a fatty acid still more unsaturated than linolic acid introduces a further factor, and the calculation would not hold good in such cases.

Eleostearin, the glyceride of the distinctive fatty acid of tung oil, gives an iodine value exactly twice that calculated from the thiocyanogen value, so that the method can be used in studying the composition of tung oil (*Ber.*, 1926, **59**, 1390). Experiments

have shown that the addition of thiocyanogen takes place at one of the three double bonds of the glyceride of pure β-eleostearic acid or of the acid itself.

A solution of 0·1*N* bromine in methyl alcohol saturated with sodium bromide was very slowly absorbed in the dark by a solution of the acid in carbon tetrachloride, and not until after an hour was a value corresponding to an addition at two double bonds obtained (*cf.* Croxford, p. 75). By exposing the mixed solutions at a distance of 50 cm. to the rays of a mercury vapour (uviol) lamp, however, an absorption corresponding with that required by three double bonds (273) is obtained, and not for some time afterwards does the substitution of bromine begin.

By using the method of calculation given above, based on the iodine values calculated from the bromine and thiocyanogen values, Kaufmann found three specimens of tung oil to have the following composition :

	I.	II.	III
Elæostearin, per cent. . . .	78·5	83·9	87·1
Olein, per cent.	22·8	7·6	13·0
Saturated acid and unsaponifiable matter, per cent. . . .	—	8·5	—

It was assumed in these calculations that the oil contained no unsaturated fatty acids other than oleic and elæostearic acids.

Calculation of Saturated Constituents of a Fat. If all the unsaturated fatty acids in a fat react with thiocyanogen in the same way, it is often possible to calculate the amount of saturated constituents from the thiocyanogen value, with close approximation to the truth. For example, if a fat contains linolic and oleic acids, each of which requires 1 molecule of thiocyanogen for saturation, the slight difference between the molecular weights (282 as compared with 280) introduces only a negligible error, and the amount of saturated constituents may be calculated by means of the formula—

$$x = 100 - 1{\cdot}158 \times \text{thiocyanogen value.}$$

Linolenic Acid. It appears that two of the three double linkages of linolenic acid are saturated in the determination of the thiocyanogen value (*Z. angew. Chem.*, 1929, **42**, 20, 73), and it is thus possible to formulate equations connecting the percentages of oleic acid (*O*), linolic acid (*L*) and linolenic acid (*Le*) with the total fatty acids (*G*) and the iodine (*I*) and thiocyanogen values (*T*).

In applying the method to a linseed oil, the sample is saponified (with precautions to prevent oxidation), the unsaponifiable matter separated, and the fatty acids liberated, extracted with petroleum spirit, and dried over freshly ignited sodium sulphate. The iodine value is then determined by means of *N* 10 bromine solution (*but cf.* Croxford, p. 75), and the thiocyanogen value upon a separate portion, a large excess (200 per cent.) of a 0·13*N* solution of the reagent being used and an absorption of twenty-four hours being given. The unsaturated fatty acids are determined, preferably by means of Bertram's oxidation method (p. 63), and the respective amounts of acids then calculated by means of the equations—

(i.) $G + O + L + Le = 100$
(ii.) $89{\cdot}93\,O + 181{\cdot}14\,L + 273{\cdot}7\,Le = 100\,I$
(iii.) $89{\cdot}93\,O + 90{\cdot}57\,L + 182{\cdot}46\,Le = 100\,T$.

The saturated fatty acids, *G*, are found by means of the equation

$$G = 100 - 1{\cdot}100\,T,$$

but oleic acid may be replaced by isomeric acids, such as elaidic acid, petroselinic acid, etc.

A Calcutta linseed oil thus examined was found to have the following composition: Saturated fatty acids and unsaponifiable matter, 10·8; oleic acid, 11·9; linolic acid, 32·6; linolenic acid 40·2; and glycerol residue, 4·5 per cent.

CHEMICAL TESTS FOR VITAMINS IN OILS

One of the most striking advances in connection with the chemical examination of oils and fats has been the correlation of their vitamine activity with specific chemical colour reactions. It was shown by Drummond and Watson (*Analyst*, 1922, **47**, 341) that the well-known purple coloration given by liver oils with

sulphuric acid is due to a substance which is present in the livers of different animals and appears to be associated with the accessory food factor termed "vitamin A." Then Rosenheim and Drummond (*Biochem. J.*, 1925, **19**, 753) found that arsenic chloride gives a brilliant blue coloration with cod-liver oil, which, like the sulphuric acid reaction, appears to be characteristic of vitamin A. It was found that there was a close agreement between the intensity of the colour reaction and the growth-promoting activity, as tested by experiments on animals, in the case of some thirty different oils and fats. Similar colour reactions were also observed with other reagents, and, like the arsenic chloride reaction, these appeared to be associated with the presence of vitamin A.

Carr and Price (*Biochem. J.*, 1926, **20**, 497) found that antimony trichloride is preferable to arsenic chloride for the test, since the blue coloration is more pronounced and intense, and they introduced the use of the tintometer for measuring the intensity of the colour, and so obtaining a measure of the vitamine activity.

Nokes and Willimott (*Analyst*, 1927, **52**, 515) studied the conditions for obtaining the best results in the reaction, and showed that the quantitative test should be made with strict regard to the time of taking the reading.

In 1927 a sub-committee was appointed at the International Conference of the League of Nations Health Organisation to examine and report upon the colorimetric methods in comparison with the biological method of determining vitamin A in cod-liver oil. The report, which was published in the *Lancet* (January 21st, 1928) (*Analyst*, 1928, **53**, 156), shows that the colorimetric methods afford information consistent with that derived from the biological tests.

The conditions for standardising the test are as follows :—

Arsenic Chloride Method. The oil is measured from a Wright's capillary pipette, graduated by means of mercury to hold 25 c.c. and delivering 20 mg. of oil into a test-tube of clear white glass of 10 mm. internal diameter. One c.c. of the reagent (pure $AsCl_3$) is delivered from a standard 10-c.c. burette into the test-tube, and a reading taken immediately (time limit one minute) in a Lovibond colorimeter. The results are expressed in standard units (blue) of the colorimeter. Five consecutive tests made in each case differed on the average by $\pm$ 0·1 unit.

Antimony Chloride Method. Two c.c. of the oil are measured by means of a standard pipette into a 10-c.c. measuring flask. The pipette is rinsed out three times with chloroform, and the solution made up to volume with the same solvent. The oil (0·2 c.c.) is measured into a test-tube (10 mm. diameter) and mixed with 2 c.c. of a 30 per cent. solution of antimony chloride solution in chloroform delivered from a standard burette. The readings are taken as above described. Limit of error as above.

It was found that the values obtained by the two methods agreed within the respective limits of error.

An interesting application of the colorimetric method was its use in court in April, 1928, in a summons against a firm of wholesale druggists for selling cod-liver oil tablets claimed to be rich in vitamin A, but which were found both by the chemical and biological tests to be deficient in vitamins.

Norris and Church (*J. Biol. Chem.*, 1930, **85**, 477) have made a critical study of the antimony trichloride colour reaction, and have found that saturated fatty acids and oils have no effect upon the colour obtained with the unsaponifiable matter of cod-liver oil, whereas oleic acid and unsaturated oil promote the rate of fading of the blue coloration. The red coloration developed is due to some substance other than vitamin A in unsaturated oils. In order to have a quantitative comparison between the colour values and the results of feeding tests, it is essential that the dilution curve should approximate to a linear function (which is only the case with the low values) or that the tests should be made upon the unsaponifiable matter of the oil.

The Rosenheim-Schuster Colorimeter. The measurement, by means of standard tintometer glasses, of the depth of colour produced in the arsenic or antimony colour reactions is greatly facilitated by the use of the colorimeter devised by Rosenheim and Schuster (*Biochem. J.*, 1927, **21**, 1329). This instrument is provided with a recess in which is placed a standard glass cell or test-tube of known diameter, containing the solution under examination, whilst the Lovibond standard glasses are fitted into horizontal carriers, which slide from left to right and are controlled by a series of knobs. The uppermost pair of these knobs control the red standards (units, 1·0 to 9·0; decimals,

0·1 to 0·9); the second pair the yellow standards; and the next pair the blue standards; whilst the lowest knob controls a carrier for special standards, such as the 10 and 20 red, 10 and 20 blue, etc.

When making an observation, the solution in the standard cell or test-tube is viewed through the eyepiece of the instrument, and the knobs are moved towards the right until an exact match of the colour is obtained. If the solution is brighter than the

FIG. 1.—The Rosenheim-Schuster Colorimeter. Based on Lovibond's colour system.

standard glasses which match it in tone, neutral tint standards (comprising combinations of red, yellow and blue glasses of equal value) are introduced into a slot immediately in front of the cell holder.

The readings are then taken by noting the positions of the respective knobs on the scale on the index plate, the upper of the paired knobs giving the units, and the lower the decimals.

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CHAPTER III

ESSENTIAL OILS

By Ernest J. Parry, B.Sc., F.I.C.

Determination of Physical Constants : Freezing and Melting Points—Boiling Points. *Chemical Methods :* Aldehydes and Ketones—Alcohols and other Acetylisable Constituents—Esters—Phenols—Oxides, etc.

THE greatest advance to be noted in connection with the analysis of essential oils has been the appointment of a sub-committee[1] for the express purpose of studying the various methods, physical and chemical, commonly used in the examination of these oils, and to make recommendations to the Standing Committee on Uniformity of Analytical Methods of the Society of Public Analysts on the methods found most trustworthy.

DETERMINATION OF PHYSICAL CONSTANTS

The Sub-Committee has issued two Reports on the methods of determining the physical constants of essential oils. The first of these, published in the *Analyst* (1927, **52**, 53), makes the following recommendations :—

Oils before testing should be clear at 15·5° C., and, if necessary, should be filtered through dry filter paper in a covered funnel : where this is necessary it should be stated on the certificate.

Specific Gravities should be determined at 15·5° C., water at 15·5° C. being taken as unity. The results should be calculated to the nearest 0 or 5 in the 4th decimal place.

Refractive Indices should be determined at 20° C. for the D line, and the reading to the 4th decimal place should be given.

[1] The Sub-Committee consisted of the following members :—J. Allan (Chairman), C. T. Bennett, S. W. Bradley, E. T. Brewis, L. E. Campbell, T. H. Durrans, T. W. Harrison, E. J. Parry, C. E. Sage, M. S. Salamon (since deceased), W. H. Simmons and T. T. Cocking (Hon. Sec.).

Exceptions. On essential oils with melting points above 20° C., determinations should be made at suitable higher temperatures.

Optical Rotation should be determined at 20° C. and expressed as that given by a column of liquid 100 mm. long for the D line.

Temperatures, if different from those prescribed above, should be stated on the certificate. The Committee does not recommend the use of corrections in any of the above determinations, as these are different for various oils.

In their second Report on Physical Constants (*Analyst*, 1929, **54**, 335), the Essential Oil Sub-Committee made the following recommendations:

Freezing and Melting Points

The apparatus recommended consists of a stout-walled glass test-tube, 125 mm. × 30 mm. (inside measurements), fitted into a wide-mouthed jar or bottle of about 500 c.c. capacity, by means of a bored cork; and an inner test-tube, 100 mm. × 21 mm., fitted into the larger tube also by means of a bored cork. The thermometer used should be readable to one-fifth of a degree, and should have a diameter about 5 mm. or 6 mm., and the length of the bulb should be between 15 mm. and 20 mm.

Freezing Points: Method of Procedure. In order to obtain a preliminary indication, a few c.c. of the oil are cooled in a small test-tube and stirred with the thermometer until solidification takes place; the temperature is noted and the tube of solidified oil set aside in a cool place. The outer container of the apparatus is then filled with water (or brine) at a temperature about 5° lower than that indicated above, and the larger outer tube fitted in its place. Into the inner tube 10 c.c. of the oil are placed, the thermometer inserted, and the tube and oil cooled to the temperature indicated in the preliminary test. The tube and contents are now inserted in the apparatus, and the temperature allowed to fall a further 1° or 2°. The oil is then seeded with a trace of the previously solidified oil and stirred with the thermometer until solidification takes place.

The highest temperature reached is taken as the freezing point.

Melting Points. After the determination of the freezing point the inner and outer tubes are removed together from the water jacket and the temperature allowed to rise slowly, the oil being stirred continuously with the thermometer until the liquid becomes "clear." If necessary, the temperature may be raised by holding the outer tube in the hand, or, in the case of a low melting point, the water jacket may be used to prevent too rapid a rise in temperature. The temperature at which the liquid becomes "clear" is taken as the melting point.

A few crystals usually remain unmelted at this point, and the appear-

ance of these crystals furnishes a sharp indication of the melting point. Until the liquid becomes "clear" the unmelted crystals are dull, but at the "clearing" point they suddenly become glistening.

When testing oils of low melting point, the result may be vitiated by the presence of moisture, which will prevent the "clearing" of the oil. An oil which is originally clear below its melting point may become cloudy from atmospheric moisture condensed in the tube during the cooling. In such cases the oils must be dried with anhydrous sodium sulphate.

Determinations on a number of aniseed and fennel oils by members of this Sub-Committee showed variations not exceeding $\pm$ 0·2° C.

Otto of Rose. In the case of otto of rose the freezing point cannot be determined by the standard method, as there is no definite rise in temperature on solidification. The following method is recommended in the case of this oil: The prescribed apparatus is used, the outer container having been filled with water about 10° below the freezing point, as indicated by a preliminary test. The oil is placed in the inner tube and stirred gently with the thermometer until crystals begin to separate. This point is taken as the freezing point. The temperature is allowed to fall a further 2°, and then the two tubes together are removed from the water jacket. The temperature is allowed to rise slowly, stirring gently the while until the liquid becomes free from all but a few characteristic glistening crystals. This point is taken as the melting point. Tests may differ by as much as $\pm$ 0·5° C.

Boiling Points

The Sub-Committee considers that uniformity can be attained only by the use of standardised apparatus and conditions, and the following are recommended:

(1) The shape and dimensions of the distilling flask are to be in accordance with a sketch provided in the original report.

(2) The flask is to be supported on a sheet of asbestos board through which a hole 4 cm. in diameter has been cut. Both flask and burner are to be protected from draughts by a screen, in accordance with the sketch.

(3) A plain glass tube, 1 to 1·2 cm. bore, and 65 cm. long, is to be used as an air condenser. The lower end is to be bent down and drawn out slightly. The condenser is to be connected with the delivery tube of the flask by means of a bored cork.

(4) The amount taken for the test is to be 50 c.c.

(5) The flask is to be heated by a naked flame, and a fragment of broken porcelain or pipe stem added to promote even ebullition.

(6) The rate of distillation is to be 50 to 70 drops per minute.

(7) The thermometer is to be either of the short-stem type or else corrected for emergent column, and the top of the bulb is to be level with the bottom side of the delivery tube.

(8) The temperature is to be corrected :

(i.) For variation in barometric pressure

$\pm$ 1° C. for each 20 mm. variant from 760 mm.

(ii.) For emergent column by the formula :

$$T = t + 0{\cdot}000143\,(t - t^1)N$$

where T = corrected temperature, t = observed temperature, t^1 = mean temperature of emergent column, and N = length of emergent column in scale degrees.

Thermometers. The accuracy of the thermometers used in these determinations is to be checked by comparison with N.P.L. standard instruments.

CHEMICAL METHODS

The advances which have to be recorded in the chemical methods of analysis may be conveniently surveyed under the following headings : I. Aldehydes and Ketones ; II. Alcohols (and Other Acetylisable Constituents) ; III. Esters ; IV. Phenols ; and V. Oxides, etc.

I. ALDEHYDES AND KETONES

The Detection of Aldehydes. Van Eck (*Pharm. Weekbl.*, 1923, **60**, 1204) proposes the use of benzidine as a useful reagent for the detection of aldehydes, which give very beautiful colours with this reagent. A solution of benzidine in glacial acetic acid gives the following reactions : Formaldehyde gives a faint yellow after some time ; on heating, the colour changes to cherry-red, and if the reaction is carried out with gaseous formaldehyde on a piece of filter paper soaked in the reagent, the colour is orange. Acetic aldehyde develops similar colours more quickly, but on paper the colour is carmine. Citral yields a deep yellow, so marked that the oil cells in fresh lemon peel can be identified by means of this reagent. Benzaldehyde yields a deep yellow solid compound ; cumic aldehyde, orange-coloured feathery crystals ; and anisic aldehyde, orange crystals. Vanillin gives a dark orange-

red, changing to a scarlet-red on addition of water; and cinnamic aldehyde gives a dark blood-red colour, with rhombic crystals.

The Determination of Chlorine in Benzaldehyde, etc.—The determination of chlorine in such substances as benzaldehyde, methyl salicylate, and many synthetic perfumes is of considerable importance for two reasons. Some of these substances, such as the two first-named, are commonly used to adulterate natural essential oils. These are free from chlorine, whereas the adulterants frequently, but not always, contain traces of chlorine, due to their method of manufacture. Further, the presence of more than the very faintest traces of chlorine in artificial perfumes causes discoloration or spotting in soap, etc. which has been perfumed with them.

A very satisfactory method has been devised in the laboratories of Messrs. Schimmel & Co. by Rubke (*Ber. Schimmel & Co.*, 1923, **96**, 2; *Z. angew. Chem.*, 1923, **36**, 156). It depends on the burning of a weighed quantity of the substance, absorbing the hydrochloric acid present in a dilute solution of caustic potash, and titrating the residual alkali. To carry out this most satisfactory determination, an apparatus of the following type should be used (it is stocked by F. Hujershoff, of Leipzig, who has made it to Rubke's design):

The important part of the apparatus is that used for the combustion; this consists of a flask-shaped container of about 20 c.c. capacity, with a suitable burner, over which is fixed a chimney tube with an inlet for air to be drawn through the apparatus. This chimney tube leads to the two U-tubes, in which the hydrochloric acid is absorbed. These are filled to three-quarters of their length with glass balls, and 25 c.c. of chlorine-free 1·50*N* caustic potash solution is added. A safety tube, containing a little water, is connected, on the one hand, with the second of the U-tubes (both of which have splash heads), and, on the other, with a water pump. The air drawn through must be dried by passing it through sulphuric acid and be perfectly free from chlorine (*e.g.*, no hydrochloric acid should be used in the laboratory to contaminate the air). The absorption liquid, properly collected, is neutralised with *N* 2 sulphuric acid, and titrated with 1·50*N* silver nitrate solution. The results are very exact if the flame is kept so that 1 grm. is burned per hour. The weight of the lamp, which is filled with the substance to be

tested, before and after the operation gives the amount of substance consumed.

The importance attaching to the determination of traces of chlorine in this type of compound has caused a good deal of attention to be paid to the subject, and numerous methods and modifications have been suggested. Voigt (*Z. angew. Chem.*, 1922, **35**, 654) has worked out a process depending on the combustion of the substance in a lamp using both hydrogen and oxygen. It consists of a horizontal cylindrical vessel closed at both ends, into which a hydrogen delivery tube enters near one end, whilst at the other end there is an exit tube bent up at right angles, which terminates with a quartz capillary tube in the combustion tube. Oxygen enters the combustion tube without passing through the lamp. The lamp is charged with a weighed quantity of, *e.g.*, benzaldehyde. The benzaldehyde vapour and hydrogen are burned with the oxygen stream in the combustion tube, which is charged with pure dry sodium carbonate granules. The chloride is titrated with silver nitrate solution in the usual manner. This method is cumbersome and requires expensive apparatus.

Probably Schimmel's lamp combustion is the most satisfactory where great accuracy is required.

The Determination of Ionone. Ito (*Osaka Research Lab. Report*, 1924, **5**, 10; 1925, **6**, 12) has described a method for the determination of ionone by means of its semicarbazone (*vide Chem. Abstr.*, 1926, **20**, 2847). Hendriksz and Reclaire have examined the subject exhaustively, and criticise the method described by Bayer (*Perf. and Ess. Oil Rec.*, 1928, **19**, 493).

According to this method, the ionone is boiled with a solution of sodium bisulphite and afterwards treated with ether. The ether is then removed by distillation, the non-ionone portions being weighed. This method has, however, drawbacks. In the first place it requires much time; one has sometimes to boil for fifteen hours, and it often appears that the non-ionone portions still contain ionone, so that it has again to be boiled for a long time with a solution of bisulphite. During the boiling the liquid often bumps violently, so that sometimes the flasks break. After the

boiling, extraction with ether has still to be repeated several times, and the non-ionone portions have to be weighed finally again. The method consequently takes up much time and is cumbrous. Moreover, one can only judge by odour whether all the ionone has combined with the bisulphite. If the boiling with bisulphite is repeated too much, one finds an ionone-content of 100 per cent.

They find that ionone can be determined quantitatively by the following method :

Five c.c. of ionone are "refluxed" for two hours with a mixture obtained by dissolving 15 grms. of hydroxylamine hydrochloride in 37·5 grms. of water, adding to this solution 18 grms. of potash, diluted in 37·5 grms. of water. The mixture thus obtained has to be filtered if necessary. After boiling, the mixture is poured, as hot as possible, into a separating funnel, the aqueous layer is separated, and the oximated oil is washed thrice with hot brine and filtered as hot as possible (which can be done in a little drying oven at about 100°). In about 0·5 to 1 grm. of the oximated oil the nitrogen is determined according to Kjeldahl, with Gunning's modification, the destruction being completed in most cases in two to three hours. The ionone-content is calculated according to the formula :

$$x = \frac{53{\cdot}82a}{14 - 0{\cdot}042a},$$

in which a represents the number of c.c. of $N/5$ sulphuric acid required for 1 grm. of oximated oil.

They ascertained in this way the percentage of ionone in the following samples :

(1) Ionone technical (d_{15}^{15} 0·9410 ; n_{D}^{20} 1·5008). 86·6 per cent. by oximation ; 87·2 per cent. bisulphite method.

(2) Ionone technical (d_{15}^{15} 0·9393 ; n_{D}^{20} 1·5029). 87·7 per cent. by oximation ; 71·1 per cent. bisulphite method.

(3) Ionone purified (d_{15}^{15} 0·9396 ; n_{D}^{20} 1·5031). 99·3 per cent. by oximation ; 98·7 per cent. bisulphite method.

(4) α-Ionone purified (d_{15}^{15} 0·9356 ; n_{D}^{20} 1·4992). 97·6 per cent. by oximation ; 99·3 per cent. bisulphite method.

(5) α-Ionone purified (d_{15}^{15} 0·9370 ; n_{D}^{20} 1·5011). 97·8 per cent. by oximation ; 98·4 per cent. bisulphite method.

(6) β Ionone purified (d_{15}^{15} 0·9488 ; n_{D}^{20} 1·5200). 98·1 per cent. by oximation ; 99·6 per cent. bisulphite method.

The Determination of Carvone. Reilly (*Analyst*, 1928, **53**, 209) describes a method for the determination of carvone in dill oil by the use of semicarbazide. Two isomeric semicarbazones have been described. One melts at 141° to 142°, and is obtained when the mixture of reagents is externally cooled: the other, melting at 163°, is formed if the temperature is allowed to rise.

The pure carvone used for experimental purposes was prepared from the crystalline compound with hydrogen sulphide. Two grms. of carvone dissolved in 50 c.c. of alcohol were added to a solution of 2·25 grms. of semicarbazide hydrochloride in 10 c.c. of water. To the cooled mixture 2 grms. of fused sodium acetate in 12 c.c. of water were added. The resulting mixture was allowed to stand at room temperature for about twenty-four hours, when most of the semicarbazone crystallised out. Water was then added to make the total volume 350 c.c. After standing for a short time, the precipitated semicarbazone was collected and dried in a vacuum desiccator. With 2 grms. of carvone used, the determination gave 1·93 grms.; with 5 grms. used, it gave 4·84 grms. The crude semicarbazone melted at 140° to 142°.

The method of procedure was the same with oil of dill as that described. To a solution of 6 grms. of semicarbazide hydrochloride in 15 c.c. of water, a solution of 10 grms. of the oil in 120 c.c. of alcohol was added, and then a solution of 6 grms. of fused sodium acetate in 10 c.c. of hot water. After twenty-four hours the whole was diluted to 840 c.c. with water, and the collected semicarbazone was dried over sulphuric acid *in vacuo* and weighed. The results obtained agree well with those by the neutral sulphite method, and, as the semicarbazone is pure and melts sharply, it is pretty clear that carvone is the only ketone present in the oil.

The Determination of Citral in Lemon Oil, and Citronellal in Citronella Oil. Bennett and Salamon (*Perf. and Ess. Oil Rec.*, 1927, **18**, 511) have published a note on the determination of aldehydes in essential oils, with particular reference to the determination of citronellal in Java citronella oil and citral in lemon oil. The method had been communicated to them, but the actual originator is stated to be unknown.

For the examination of citronella oil 2 grms. are weighed into a flask, and 20 c.c. of a 5 per cent. solution of hydroxylamine hydrochloride in 80 per cent. alcohol added. The mixture is titrated with *N*/2 alcoholic potash, with methyl orange as indicator.

Bromphenol blue was suggested by Bennett and Salamon as a better indicator, and they consider that the results obtained are accurate, and, in the case of lemon oil, agree substantially with the method at present official in the British Pharmacopœia.

The writer has examined some hundreds of samples of lemon oil by this process, and is definitely of opinion that it is the most accurate method of determining citral in lemon oil. The method to be followed is as follows :

Ten c.c. of the oil (calculated to its weight according to temperature) are mixed with 10 c.c. of the hydroxylamine hydrochloride solution. The mixture is vigorously shaken at frequent intervals for half an hour, in order to bring the citral into solution, and a few drops of a solution of *p*-dimethylamino-azo-benzene used as indicator. The mixture is then titrated with *N* 2 alcoholic potash, and the citral calculated in the usual manner. A little experience is necessary for the correct observation of the end-point ; this process yields results which are probably more accurate than those obtained by any other process hitherto published.

The Essential Oil Sub-Committee to the Standing Committee on Uniformity of Analytical Methods has made the following report on this question (*Analyst*, 1930, **55**, 109) :—

The Determination of Citral in Lemon Oil

The Essential Oil Sub-Committee has found that the method official in the British Pharmacopœia, 1914, for the determination of citral in lemon oil is open to objection on account of the large variation in the results obtained.

Numerous experiments have been carried out, and, as a result, the Sub-Committee unanimously recommends the method given below. To ensure a reasonably sharp end-point being obtained, it is essential that strict attention be paid to the relative proportions of the reacting substances.

The following solutions are required :—

Indicator Solution. A 0·2 per cent. solution of pure methyl orange in 60 per cent. alcohol.

N 2 *Alcoholic Potash.* Prepared with pure 60 per cent. (by volume) alcohol and standardised against *N* 2 hydrochloric acid, using methyl orange as indicator, and running the alkali *into* the acid until the full yellow colour is obtained.

N 2 *Hydroxylamine Hydrochloride.* Dissolve 3·475 grms. of pure hydroxylamine hydrochloride in 95 c.c. of pure 60 per cent.

(by volume) alcohol; add 10 drops of the indicator solution, adjust to the full yellow colour of the indicator with the *N* 2 alcoholic potash, and make up to 100 c.c. with 60 per cent. (by volume) alcohol.

Method of Determination. Weigh out exactly, into a stoppered tube approximately 150 mm. long by 25 m.m. diameter about 10 grms. of the lemon oil, add 7 c.c. of *N* 2 hydroxylamine hydrochloride and 1 drop of the indicator: shake and titrate with the *N* 2 alcoholic potash until the red colour changes to yellow. Continue the shaking and titrating until the full yellow colour of the indicator is permanent in the lower layer after shaking vigorously for two minutes, and then allowing it to separate. The reaction is slow towards the end, but should be complete in about fifteen minutes.

The completed titration liquid, plus a slight excess of alcoholic potash (1 or 2 drops), may be used as colour standard for the end-point of a subsequent determination.

The number of c.c. of *N* 2 alcoholic potash required, multiplied by the correcting factor 1·008, by the factor 0·076 for citral, and by 100, and divided by the weight of the oil taken, will give the percentage of aldehydes, calculated as citral.

The correcting factor is necessary, owing to the fact that the end-point of the titration, as carried out above, occurs at a *p*H different from that of neutral hydroxylamine hydrochloride.

The volume of the *N* 2 hydroxylamine hydrochloride used should be varied according to the citral content of the oil, the excess of hydroxylamine hydrochloride over the alcoholic potash required, being from 1 c.c. to 2 c.c. in all cases.

Determinations made by members of the Sub-Committee led to the opinion that the maximum variation with this process is within ±0·1 per cent.

Results of the Determination of Citral in a Sample of Lemon Oil by Members of the Sub-Committee.

Member.			Member.	
(1)	4·04 per cent.	..	(7)	4·03 per cent.
	4·05 „	..		3·96 „
(2)	3·89 „	..	(8)	4·05 „
(3)	3·95 „	..	(9)	4·05 „
(4)	4·03 „	..		4·05 „
	3·95 „	..		4·05 „
(5)	4·08 „	..	(10)	3·94 „
(6)	4·07 „	..		4·01 „

Maximum variation 0·19 per cent.

Penfold and Armeman have published a paper (see *Perf. and Ess. Oil Rec.*, 1929, **20**, 392) on the determination of citronellal and citral in essential oils, and prefer the following modification of the method just described (which appears to be due to Holtappel) :

About 2 grms. of oil are cooled in a mixture of salt and ice. Twenty c.c. of 5 per cent. hydroxylamine hydrochloride, containing the bromphenol blue indicator, and 30 c.c. of *N* 2 alcoholic potash are mixed and similarly cooled to a temperature of − 10°. The two solutions are then mixed, the flask holding the potash mixture being washed out with rectified spirit. This is allowed to stand for fifteen minutes in the brine bath and then allowed to attain room temperature. The excess potash is determined by titration with *N* 2 alcoholic hydrochloric acid solution.

The whole determination can be carried out within an hour. The end-point is very easily observed, the solution changing from blue through green to yellow within a few drops of acid. It was found advisable, when the green stage was reached, to allow the solution to stand a few seconds to enable the observer to pick out the change from green to yellow. The percentage of citronellal is calculated from the following equation :

$$\text{Per cent. of citronellal} = \frac{100 \times 0{\cdot}077 \times C}{W}$$

where C = c.c. *N* 2 alcoholic KOH used, and W = weight of oil taken

The writer has examined many samples of lemon oil by the cold hydroxylamine process, and there is certainly no need to use a freezing mixture in the case of citral. Di-methyl-aminoazobenzene is sharper in end-reaction than bromphenol blue.

Radcliffe and Swaren (*Perf. and Ess. Oil Rec.*, 1928, **19**, 17) have suggested a method for the determination of citral in lemon oil, which, although it may yield good results, has no advantage over the hydroxylamine process, and is considerably more tedious and liable to errors.

The principle of this method is to add a known excess of pure thiosemicarbazide (m.p. 180° to 182°) to a known amount of the essential oil, commercial aldehyde or ketone, to form the thiosemicarbazone. The excess of thiosemicarbazide is extracted by a suitable solvent and weighed. From the difference, *i.e.*, the reacting quantity, the weight of aldehyde or ketone can be calculated.

The following precautions are necessary : The solvent should be of low boiling point and should leave the extracted thiosemicarbazide after reaction in a pure state, as shown by its mean percentage ; the thiosemicarbazide should be pure, the oil free from suspended matter, the diluent alcohol free from aldehyde or ketone, and the weighed filter for collection of excess thiosemicarbazide should be unaffected by the solvent.

Each of six samples of lemon oil was examined in duplicate by the following method :

From 2 to 3 grms. of oil were accurately weighed into a 250-c.c. conical flask, and 0·8 grm. of pure thiosemicarbazide, also accurately weighed, added with about 50 c.c. of alcohol. The flask was placed on a water bath until most of the thiosemicarbazide had dissolved, after which the liquid was evaporated to dryness. The residue consisted of citral thiosemicarbazone, excess thiosemicarbazide, and some impurity from the oil. This was dried by passing air over the contents of the flask, immersed in boiling water. About 150 c.c. of pure carbon disulphide were then added, and the contents of the flask boiled for a few minutes to dissolve the thiosemicarbazone. The solution was filtered through a weighed filter, and the excess of thiosemicarbazide well washed with carbon disulphide. Any thiosemicarbazide tending to adhere to the side of the flask was scraped off and washed onto the filter with carbon disulphide. Exposure of the filter in the funnel to the air removed most of the solvent. Drying was completed in a steam oven at 50°, the funnel cooled in a desiccator, and the paper removed and weighed. This gave the excess thiosemicarbazide, which, subtracted from that taken, gave the quantity for the formation of thiosemicarbazone : multiplying this by 1·67, the amount of citral in the weight of oil taken was obtained.

II. ALCOHOLS AND OTHER ACETYLISABLE CONSTITUENTS)

Process of the Essential Oil Sub-Committee. The variations in the methods used in acetylation processes for essential oils have been examined by a special sub-committee appointed by the Standing Committee on Uniformity of Analytical Methods, and the Sub-Committee has made an interim report (*vide Analyst*, 1928, **53**, 214). The report is as follows :

As a result of the investigations of the Essential Oil Sub-Committee, evidence has accumulated that even slight variations in the procedure

adopted by analysts in carrying out the determination of the acetylisable constituents of essential oils are responsible for serious differences in the results obtained.

The Sub-Committee feel that it is urgently necessary to avoid these differences, and, after extended investigations, the following detailed method of procedure has been agreed upon and is presented with this end in view :

Method of Acetylation. Ten c.c. of the oil, 20 c.c. of acetic anhydride (95 to 100 per cent.), and 2 grms. of freshly fused anhydrous sodium acetate are mixed in a long-necked, round bottomed 200-c.c. Kjeldahl flask ; a fragment of broken glass is added, and the contents boiled gently under an air reflux condenser for two hours.

The flask should be supported on a sheet of asbestos board, in which has been cut a hole about 1½ inches in diameter, and should be heated by a small naked flame, placed about 1 inch below, and not impinging on the bottom of the flask.

At the expiration of two hours, the flame is removed and the flask allowed to cool ; 50 c.c. of water are added, and the flask and contents heated on a boiling water bath for fifteen minutes, with frequent and thorough shaking. After cooling, the contents of the flask are transferred to a separating funnel and the lower aqueous layer rejected. The acetylated oil is then washed successively with : (1) 50 c.c. of brine (saturated aqueous solution of sodium chloride) ; (2) 50 c.c. of brine containing 1 grm. of sodium carbonate in solution ; (3) 50 c.c. of brine ; (4) 20 c.c. of water. Mixtures 1, 2 and 3 should be shaken vigorously, but the final washing with water must be conducted with gentle shaking only.

If the washing operations have been properly conducted, the aqueous layer from the second washing should be alkaline to phenolphthalein. (Alcoholic phenolphthalein must not be added to the mixture in the separator.)

When the washing is complete, the aqueous layer is removed as completely as possible, and the oil poured out and mixed with about 3 grms. of powdered anhydrous sodium sulphate, stirred for fifteen minutes or until 1 drop of the oil produces no cloudiness when added to 10 drops of carbon disulphide in a dry test-tube. The oil is then filtered through a dry filter paper in a covered funnel.

Method of Hydrolysis. About 2 grms. of the dried and filtered oil are accurately weighed into a hard glass flask, 2 c.c. of distilled water added, and the free acidity titrated with *N* 10 *aqueous* KOH, with 1 c.c. of 1 per cent. solution of phenolphthalein in 60 per cent. alcohol as indicator. Forty c.c. of *N* 2 alcoholic KOH are then added and the mixture boiled under a reflux condenser on a water bath for one hour :

the flask is then cooled rapidly, 20 c.c. of distilled water added, and the excess of alkali titrated with N 2 H_2SO_4.

A blank determination of the alcoholic potash must be carried out simultaneously with the hydrolysis of the acetylated oil, and under conditions conforming as nearly as possible with those employed therein.

The Differentiation between Geraniol and Citronellal in Citronella Oil. The manufacture of hydroxycitronellal has necessitated a method for the accurate determination of citronellal in citronella oil, instead of the usual determination of total acetylisable constituents returned as geraniol. According to Dupont and Labaune (*Chimie et Industrie*, August, 1924), this may be done as follows :

(1) Ten grms. of citronella oil, 10 grms. of acetic anhydride, and 2 grms. of fused sodium acetate are boiled for ninety minutes, and the total acetylisable constituents determined in the usual manner. This may be taken as the sum of the geraniol and citronellal.

(2) A solution of 10 grms. of hydroxylamine hydrochloride in 25 c.c. of water is neutralised by the addition of a solution of 10 grms. of sodium carbonate in 25 c.c. of water. This mixture is added to 10 grms. of the citronella oil, and the whole warmed to 20° to 25° with constant agitation. The supernatant oil is separated, washed with water, dried and boiled with acetic anhydride for two hours. The total acetylisable constituents in this mixture, separated, washed and dried in the usual manner, are now determined. The geraniol alone is esterified, the citronellal oxime being converted into nitrile. The amount of "alcohols," as determined on the oil, minus those found in the oximated oil, gives the amount of citronellal.

Reclaire and Speelstra (*Perf. and Ess. Oil Rec.*, 1927, **18**, 130) consider that the formation of citronellal diacetate during acetylation is responsible for differences in the determinations of the "total alcohols" in citronella oil, the amount formed varying according to the conditions of the acetylation. They adopt the principles of Dupont and Labaune, but prefer to determine the nitrogen in the oximated oil by the Kjeldahl method, and so calculate the citronellal, which can then be deducted from the total acetylisable constituents.

The Determination of Linalol and Terpineol. Boulez (*Bull. Soc. Chim.*, 1924, [iv.], **35**, 419) has suggested the following

modification of his original method for the determination of linalol, which cannot be carried out by the ordinary acetylation processes :

In a flask of 250 c.c. capacity 1 grm. of the sample and 22 grms. of *m*-xylene are weighed, and 10 grms. of acetic anhydride and 3 grms. of fused sodium acetate are added, and the mixture boiled for about nine hours. When cooled, 50 c.c. of water are added, and the mixture heated for half an hour on the water bath. The xylene solution is separated from the aqueous layer and washed once with hot water. It is then dried over anhydrous sodium sulphate, filtered, and the ester value determined on about 5 grms. of the filtrate. (The obvious objection to the details of this process are that the final determination is made on as little as 0·25 grm. of the original substance, so that the error may be very large).

Glichitch (*Compt. rend.*, 1923, **177**, 268) states that accurate results for the estimation of linalol and terpineol may be obtained by the following process :

Ten c.c. of the oil are mixed with 15 c.c. of a mixture of formic acid (sp. gr. 1·22) 1 part, and acetic anhydride 2 parts. The whole is well shaken and placed on ice. It is then slowly allowed to regain room temperature and is kept for seventy-two to ninety-six hours at about 20°. Excess of reagent or long reaction time does not impair the result. The excess of acid is removed by the addition of 50 c.c. of water, which remains in contact with the mixture for two hours. The oil is then washed with a solution of sodium bicarbonate, and again with water, dried by anhydrous sodium sulphate and saponified in the usual manner. Schimmel & Co. (*Report*, 1924, 121) do not consider this method accurate, and prefer the process originally suggested by Boulez, *i.c.*, by dissolving the oil in xylene (1 in 5) and acetylating in the usual manner, allowing five hours for the acetylation for terpineol and seven hours for linalol.

The Detection of Benzyl Alcohol. Benzyl alcohol has been found as an adulterant of essential oils, which naturally contain a high amount of acetylisable constituents, such as sandalwood oil. St. Pfau (*Perf. and Ess. Oil Rec.*, 1925, **16**, 190) detects this adulterant, if present in sufficient quantity, by placing 0·03 grm. of powdered dry potassium carbonate in a test-tube with 10 drops of the fraction of the oil, which should contain benzyl alcohol (or more if

the benzyl alcohol content is less than 50 per cent.), and 5 drops of diethyl oxalate. The mixture is warmed, and when the contents of the tube solidify or become yellow the tube is cooled and 2 c.c. of water added. The tube is then warmed until the contents become liquid and dibenzyl oxalate separates out as a solid crust. This is separated and washed with a little alcohol, and will then be found to melt at 79° to 80°.

The Determination of Geraniol and Citronellol.—It cannot be said that any exact method for the determination of the two alcohols, geraniol and citronellol, when present in the same essential oil, exists. At one time it was thought that the formylation process was an accurate one for this purpose, as, by heating citronellol with formic acid, the alcohol is esterified, whilst other alcohols related to it are dehydrated and converted, in the main, into terpenes. It is now recognised, however, that the conversion of geraniol into terpenes is only partial, and a certain amount of geranyl formate is formed. It is, however, possible to obtain useful results of a comparative nature as to the relative amounts of the two alcohols in samples of otto of rose, geranium oil, etc.

To 10 c.c. of the oil in question 10 c.c. of formic acid (100 per cent.) are added, and the mixture boiled for an hour under a reflux condenser. The mixture is cooled, 100 c.c. of water added, and the separated oil is washed and dried as in the ordinary acetylation process. The citronellol is calculated from the saponification figures in the usual manner:

$$\text{Citronellol percentage} = \frac{x \times 15{\cdot}6}{w - x(0{\cdot}028)}$$

where x represents the number of c.c. of normal potash solution and w the weight of the formylated oil used.

According to St. Pfau (*J. prakt. Chem.*, 1921, **102**, 276), the formylation of citronellol results in the formation of a small amount of terpenes, citronellyl formate, the mono- and di-formates of citronellol-glycol, and some polymerised residue (see also Glichitch, *Les Parfumes de France*, 1923, **6**, VII., for details of a formylation process).

Determination of Alcohols and Phenols by Means of Magnesium Methyl Iodide.—This method depends on the reaction between

magnesium methyl iodide and compounds containing the hydroxyl group, as follows :

$$R.OH + CH_3.Mg.I = CH_4 + RO.Mg.I$$

From the volume of methane evolved, the amount of the alcohol or phenol is calculated. Zerevitinoff (*Z. anal. Chem.*, 1926, **68**, 321) has shown that, whilst alcohols and phenols react quantitatively with magnesium methyl iodide, ketones, such as camphor, fenchone and menthone, give no evolution of methane. Certain ketones not found to any extent in essential oils do react, but scarcely come into consideration. If free acids are present the acid value must be determined, and its hydroxyl value allowed for in calculating the results. From 0·2 to 0·3 grm. of the dried oil should be dissolved in 15 c.c. of toluene or xylene, and the methane evolved on treatment with magnesium methyl iodide measured in a suitable apparatus for gas measurement. The calculation is made, for example, with geraniol, as follows :

$$\text{Per cent. of } C_{10}H_{18}O = \frac{0{\cdot}000719V \times 154 \times 100}{16S} = 0{\cdot}692\ \frac{V}{S}$$

where V is the volume in c.c. of methane reduced to 0° and 760 mm., and S is the weight of the oil used.

This method is too complicated for general use, but is of theoretical importance, and may be of use in the case of such alcohols as linalol which are partly decomposed by the acetylation process. For example, Zerevitinoff found 73 per cent. of linalol by this method, as against the admittedly too low figure of 66 per cent. by the acetylation process.

Determination of Primary Alcohols by Means of Phthalic Anhydride. Schimmel & Co. (*Ber.*, 1912, 39) showed that primary alcohols, such as geraniol, santalol, etc., could be determined by heating about 2 grms. of the oil, dissolved in 2 c.c. of benzene, with 2 grms. (accurately weighed) of phthalic anhydride, as in the acetylation process. When cold, the mixture is well shaken with 60 c.c. of seminormal potash solution for ten minutes. The residual phthalic anhydride is converted into neutral phthalate of potassium, and the acid ester of the alcohol into its potassium salt. The difference between the amount of potash required for

neutralisation of the same amount of phthalic anhydride (as a blank) and that required for the reaction product enables the amount of the geraniol, etc., to be calculated.

Radcliffe and Chadderton (*Perf. and Ess. Oil Rec.*, 1926, **17**, 352) recommend using 2 to 3 grms. of the oil to be examined, and 25 c.c. of a solution of 50 grms. of phthalic anhydride in 250 c.c. of pyridine. The mixture is kept at water-bath temperature for eighteen hours, and, on cooling, is diluted with water and titrated as usual with alkali. It is stated that the results are from 2 to 2·4 per cent. too low with geraniol, and 3·1 to 3·4 per cent. too low with citronellol.

Identification of Phenyl-ethyl Alcohol in Essential Oils.—Sabetay (*Ann. Chim. Anal.*, 1929, **11**, 193) describes the following method for the identification of primary phenyl-ethyl alcohol in essential oils and mixtures of perfumes:

The fraction boiling at about the range of phenyl-ethyl alcohol is slowly distilled with coarsely powdered caustic potash, and the fraction coming over at 140° to 160° is collected. This consists of styrolene, which is easily recognised by its odour if phenyl ethyl alcohol is present. This is confirmed by converting it into its di-bromo derivative, which melts at 72°. The presence of geraniol or rhodinol (citronellol?) does not interfere, unless in considerable excess. This reaction is particularly useful in perfumery practice, in dealing with synthetic alcohols, the boiling points of which are close together, and the quantity of styrolene dibromide obtained gives an approximate measure of the phenyl-ethyl alcohol originally present.

III. ESTERS

The Detection of Artificial Esters in Essential Oils.—The general principles underlying the detection of artificial esters in essential oils may be summarised as follows: (1) The difference in the time necessary for saponification under given conditions; (2) differences in solubility of esters, enabling them to be partly separated; (3) the volatility or non-volatility of the acid constituent of the esters. The only ester used in practice which is much more highly resistant to saponification than the natural esters present in the adulterated oil is terpinyl acetate, which shows a very marked increase in saponification value as between thirty minutes and

two hours, whereas the difference with such esters as linalyl acetate is negligible (*Schimmel's Report*, October, 1910, 60). Difference in solubility enables glyceryl acetate to be detected, since extraction with dilute alcohol removes the glyceryl acetate, but not the natural esters. Schimmel & Co. (*Report*, October, 1910, 61) extract 10 c.c. of, *e.g.*, bergamot oil in a separator, first mixed with 10 c.c. of petroleum spirit, with 2·5 c.c. of alcohol and 20 c.c. of water. Ten c.c. of the separated aqueous solution are neutralised and then boiled for an hour with *N* 2 alkali. The pure oil will not require more than 0·1 c.c. for this saponification, whilst each 1 per cent. of glyceryl acetate requires more than 0·5 c.c. of *N* 2 alkali.

Salamon and Seaber (*Per. and Ess. Oil. Rec.*, 1912, **3**, 275) find that 5 per cent. alcohol will extract the glyceryl acetate.

For the detection of glyceryl acetate, Coen (*J. Soc. Chem. Ind.*, 1915, **34**, 851) recommends that 10 c.c. of the oil be treated with 40 c.c. of 10 per cent. alcohol. The mixture is evaporated to a small volume, neutralised, saponified with alcoholic potash and evaporated. The residue is then extracted with a mixture of alcohol and ether, and the resulting extract is heated with potassium bisulphate. If triacetin is present acrolein is formed. Care must be taken not to drive off the glycerin by overheating in the evaporation.

Schimmel & Co. (*Report*, October, 1910, 61) suggested the determination of the amount of alkali required for saponification of the oil, and also that of the free acids obtained by steam distillation after acidifying the saponification liquid with sulphuric acid and after driving off the alcohol. With most natural esters the acids are completely volatile with steam, whereas many of the artificial esters used as adulterants are esters of fixed fatty acids, and any material difference between the two values indicates the presence of an ester or esters of fixed acids. A critical study of this method has been made by J. C. Umney (*Perf. and Ess. Oil. Rec.*, 1914, **5**, 116) and he has described standard apparatus for obtaining the best results.

C. T. Bennett and Garratt (*Perf. and Ess. Oil Rec.*, 1923, **14**, 359) give the following as a satisfactory test for many of the esters of

non-volatile acids, which depends on the insolubility of the potassium salts of the acids in absolute alcohol:

One c.c. of the oil is mixed with 3 c.c. of an approximately 10 per cent. solution of potassium hydroxide in absolute alcohol, in a test-tube, which is immersed in a water bath for a few minutes and then allowed to cool. The absence of any precipitate within one hour indicates that the oil is free from such adulterations.

The following details are recorded:

(*a*) Phthalic acid esters. One per cent. gives a crystalline precipitate almost at once.

(*b*) Cinnamic acid esters. With 2·5 per cent. a precipitate forms quickly; with 1 per cent., crystals are deposited after a time.

(*c*) Succinic acid esters. With 2·5 per cent., a gelatinous mass is formed; with 1 per cent., a crystalline precipitate is formed.

(*d*) Citric and tartaric acid esters. Down to 1 per cent. a heavy cloudiness is produced, and a crystalline deposit is formed on standing.

(*e*) Benzoic acid esters. With 2·5 per cent., a crystalline deposit forms after standing for some time.

Ethyl laurate is not detected by this method.

Reckaru (*Perf. and Ess. Oil Rec.*, 1923, **14**, 293) states that in the detection of esters of non-volatile acids, as proposed by Schimmel & Co., the evaporation of the saponification liquid may be omitted, and the acidification and distillation proceeded with directly after the determination of the saponification value. Phosphoric acid is preferred to sulphuric acid as the acidifying medium, as originally proposed by J. C. Umney.

Glyceryl acetate is so easily washed out of an essential oil by hot distilled water that an oil so adulterated, and washed with distilled water several times, will show a distinctly lower ester value and refractive index than the original washed oil (Parry, *The Chemistry of Essential Oils*, 4th Ed., Vol. II., p. 315).

Ethyl laurate is a not uncommon adulterant of bergamot and lavender oils, and if Reckaru's modification is employed, the oily liquid may dissolve the lauric acid, which is slowly but completely volatile, so that this ester might thus escape observation. By

evaporating the liquid before acidification, the lauric acid will be seen floating as oily drops on the surface of the distillate.

Romeo (*Rivista Italiana delle Essenze e Profumi*, January 15th, 1927) gives the following method for the determination of ethyl laurate :

Ten c.c. of essential oil are saponified for one hour with 50 c.c. of *N* 2 alcoholic potassium hydroxide ; when cool, the mixture is first neutralised with dilute sulphuric acid, 2 drops of phenolphthalein solution being used as indicator, then rendered slightly alkaline by the addition of a few drops of *N* 2 potassium hydroxide, whereupon it is evaporated to dryness on a water bath. The residue, while hot, is treated with 20 c.c. of water ; the whole is transferred to a small separator and rendered distinctly acid by the addition of dilute sulphuric acid, whereupon 40 c.c. of ether are added and the mixture gently shaken. The acid liquid is removed ; the ethereal solution is shaken three times with a small amount of water and then filtered into a tared capsule, and the separator and filter are rinsed with ether, which is also added to the contents of the capsule. The liquid is then evaporated to dryness, and the weight of the residue is determined. It is then dissolved in alcohol (96 per cent.), and its acid value determined by the addition of 10 c.c. of *N* 2 solution of potassium hydroxide and titration with *N*/2 sulphuric acid.

On evaporation on the water bath, a pure oil of bergamot yields 4·75 to 6·2 per cent. of residue, with a saponification value of 136 to 190. In the case of an oil adulterated with ethyl laurate, after saponification and removal of the volatile portion, the weight of the residue obtained on evaporation of the ethereal extract will be greater than that yielded by a pure oil, the weight being proportional to the amount of ethyl laurate present. Further, since the acid value of lauric acid is 280, the acid and saponification values of this residue will be higher than that shown by the residue obtained from a pure oil. With a pure oil, the difference between the index of volatile acids and the saponification value should not exceed 10.

According to Romeo, the Chemical Bureau of the United States Customs adopts the following method for testing oil of bergamot :

Twenty c.c. of the oil under examination are distilled with steam, to separate the esters of volatile fatty acids (used as adulterants) from the fixed matter naturally present in oil of bergamot, the latter not being volatile with steam. The oily distillate is saponified with *N* 2 alcoholic potassium hydroxide on a water bath for one hour, neutralised with hydrochloric acid, and evaporated to dryness : the residue is treated

with water, filtered and solution of calcium chloride added to the filtrate.

For the quantitative determination, the insoluble soap is transferred to a large filter and washed with water until the washings are neutral and the substance has become colourless, whereupon it is transferred to a separator. After acidification with hydrochloric acid, the precipitate is extracted with three portions of ether, which, after washing, are evaporated in a weighed capsule. Alcohol is added to separate the water, after which the residue is placed over-night in a desiccator. The weight of the residue, multiplied by 1·14, gives the percentage of ethyl laurate.

Romeo, however, points out that this method, when tested on absolutely genuine oil of bergamot obtained from mature as well as from immature fruits, fails to yield strictly accurate results, since a slight precipitate is always produced on treatment with solution of calcium chloride; only when it is weighable (over 0·01 grm.) does it point to adulteration with ethyl laurate.

The Saponification of Isovaleric Esters. Reclaire (*Deutsche Parf. Z.*, 1924, **10**, 189) has examined the conditions of the saponification of a number of these esters, and finds that those of isoamyl, ethyl, benzyl, and cinnamyl alcohols, and of geraniol, octanol, dinodecanol, phenyl-ethyl alcohol and santalol are saponified as readily as those of most acetates and butyrates. The isovalerates of citronellol, borneol and menthol are much less easily saponified. He recommends the use of normal alcoholic potash (20 c.c. for 1·5 grm. of ester) and two hours for the time of saponification where isovalerates are known to be present. In the case of bornyl and menthyl isovalerates, he recommends three and six hours' saponification, respectively.

Phthalic Acid Esters. The detection of phthalic esters is best carried out as follows, as recommended by R. E. Andrew (*J. Ind. Eng. Chem.*, 1923, **15**, 838):

To 10 c.c. of the solution obtained from the dried saponification residues 5 drops of a 10 per cent. solution of caustic soda are added. The mixture is evaporated to dryness, and 0·5 c.c. of a 5 per cent. solution of resorcinol is added and the mixture again evaporated to dryness. To the warm residue 6 drops of concentrated sulphuric acid are added, and the whole well mixed. When cold, 10 c.c. of water are added and the solution transferred to a test-tube, and 5 c.c. of a 10 per

cent. solution of caustic soda added. In the presence of phthalic acid a yellow-green to green fluorescence will appear.

According to Breithut and Apfelbaum (*J. Ind. Eng. Chem.*, 1925, **17**, 5), the replacement of resorcinol by phenol is a more certain test. The residue containing phthalate is treated with 5 or 6 drops of concentrated sulphuric acid and kept at 160° for three minutes. One c.c. of water is added to the fused mass, and the whole made alkaline with caustic soda solution. The characteristic pink colour, due to the presence of phenolphthalein, results, and is discharged on acidifying if phthalic acid is present.

The Determination of Methyl Anthranilate. R. D. Scott (*J. Ind. Eng. Chem.*, 1923, **15**, 732) has examined Erdmann's method for the determination of methyl anthranilate (based on the formation of insoluble azo dyes from the ester [see *Berl. Ber.*, 1902, **35**, 24]). He comes to the following conclusions:

(1) Accurate results can only be obtained when the amount of alkali present is adjusted so that it is equal to, or only very slightly in excess of, that required theoretically. The alkali used should be sodium bicarbonate, and not the carbonate or hydroxide.

(2) To combine with a given amount of β-naphthol, about 5 per cent. in excess of the theoretical amount of diazotised methyl anthranilate is required, whilst if α-naphthol is used the amount is almost theoretical.

(3) The time of diazotisation should not be long continued: Scott considers one minute sufficient.

For small quantities of methyl anthranilate, the writer prefers colorimetric determinations in Nessler glasses rather than titration.

IV. PHENOLS

The Determination of Phenols in Essential Oils. The practically universal method for the determination of phenols in essential oils is the absorption of the phenolic bodies by an aqueous solution of caustic alkali, and reading off the unabsorbed oil in the graduated neck of the flask. The details of the method have been carefully studied by the Essential Oil Sub-Committee to the Standing

Committee on Uniformity of Analytical Methods, who have reported thereon as follows (*Analyst*, 1928, **53**, 1928):

They are unanimous in opinion that the most suitable method for general purposes for the determination of phenols in the oils of ajowan, bay, cinnamon leaf, clove, origanum, pimento and thyme is that of shaking the oil with cold aqueous potassium hydroxide solution and measuring the amount of unabsorbed oil. It has been found that, in order to obtain uniform results, standard conditions must be strictly adhered to.

The determination is carried out in a flask consisting of a bulb of about 150 c.c. capacity with a long neck, 10 c.c. of which are graduated in one-tenths of a c.c., the length of the graduated portion being not less than 15 cm. Before use, the flasks should be cleansed with strong sulphuric acid and well rinsed out with distilled water.

Method of Determination. Eighty c.c. of 5 per cent. aqueous potassium hydroxide solution are placed in the flask, followed by 10 c.c. of the clear oil, and the mixture thoroughly shaken at five-minute intervals during thirty minutes, at room temperature.

The unabsorbed portion of the oil should then be raised into the neck of the flask by the gradual addition of more of the potassium hydroxide solution, and the separation of the oily layer facilitated by rotating the flask between the hands and gently tapping. After standing for not less than twenty-four hours, the volume of unabsorbed oil should be read off, taking the bottom line of the meniscus in each case. The proportion absorbed, multiplied by 10, will give the percentage by volume of the phenolic content of the oil under examination.

Where a small quantity, not exceeding 0·4 c.c., of emulsion is formed between the oily and aqueous liquids, a mean reading of this should be taken. If an emulsion is formed which will not separate, a repeat test should be carried out with the addition of 2 c.c. of xylene * to the test mixture before the initial shaking. This facilitates the separation of the oil: the final reading of unabsorbed oil should be corrected for the added xylene.

The potassium hydroxide solution should be clear and adjusted to contain 5 grms. (4·9 to 5·1) potassium hydroxide in 100 c.c.; and whilst the presence of chloride or carbonate does not materially affect the

* Xylene or xylol of commerce with a boiling range of 137° C. to 142° C. is here intended. It should be tested to ensure freedom from impurities soluble in 5 per cent. aqueous potassium hydroxide solution.

result, yet it is advisable to restrict the amount of these impurities to the proportions which are ordinarily present in good commercial stick caustic potash. It should be free from more than traces of silica and alumina, as these are detrimental impurities, giving rise to separation of flocculent matter.

Experiments have been carried out in order to determine :

(1) The best strength of alkali for absorption.

(2) The more suitable—potassium hydroxide or sodium hydroxide.

(3) Which gives the better results—hot or cold treatment.

For this purpose, mixtures of redistilled eugenol and clove terpenes ; thymol and thymene ; and carvacrol and paracymene, were employed.

The results obtained indicate that potassium hydroxide is better than sodium hydroxide, that a 5 per cent. solution is the most suitable strength for general purposes, and that, for the above oils, cold treatment is preferable.

Sodium hydroxide does not give good separations.

Small variations in the size of the flasks used made no appreciable difference in the results obtained.

The process has been found to give accurate results with known mixtures of pure phenols and terpenes. With samples of normal oils which were circulated amongst the members of the Sub-Committee, the results of the tests lead them to the opinion that the limits of error are within ± 1 per cent. This degree of accuracy cannot always be attained with oils that are oxidised, polymerised, or otherwise unusual in character

Bay Oil—With this oil a secondary liquid layer frequently occurs at the bottom of the separated non phenols. This should be included in the unabsorbed portion.

Cinnamon Leaf Oil. A series of experiments was carried out in order to determine the effect of the presence of aldehydes on the results obtained by absorption with 5 per cent. potassium hydroxide solution. It was found that the addition of as much as 10 per cent. of cinnamic aldehyde to a cinnamon leaf oil did not appreciably affect the determination of phenols by this method.

Clove Oil.—In the case of this oil, eugenol and aceto-eugenol are both absorbed.

A number of oils were tested by all members of the Sub-Committee by the hot method, as well as by the cold method. The results of tests on three typical oils by the proposed method are given below, and these show a maximum variation of 2 per cent.

Member.	Bay Oil.	Cinnamon Leaf Oil.	Thyme Oil.
(1)	50	78·5	69
(2)	49·5	78·5	67·5
(3)	48	78	68
(4)	49	79	69·5
(5)	49	79	67·5
(6)	49·5	79	68·5
	48·5	—	—
(7)	49	—	—
(8)	50	78·5	68·5
	—	78·7	—
(9)	49·5	77	68
(10)	49	78	69
(11)	50	78	68

The Determination of Eugenol.—Ghelutch (*Les Parfums de France*, 1924, 40) considers that the determination of eugenol in cinnamon leaf oil by means of caustic soda solution should be carried out in the cold, since, when heated, the esters are saponified, and combined phenols or alcohols are included in the result. For oils with a low content of eugenol, he recommends the addition of petroleum spirit to the mixture, as the non-phenolic portion usually has a higher specific gravity than the aqueous solution; it therefore settles out at the bottom of the flask, and so cannot be measured.

Differentiation and Detection of Phenols.—A. H. Ware (*Quart. J. Pharm.*, 1929, **2**, 249: *Analyst*, 1929, **54**, 614) gives the following details of tests for phenols:

(1) The phenol (0·05 grm.) is dissolved in 3 c.c. of concentrated sulphuric acid, 1 drop of a 10 per cent. solution of dihydroxyacetone

(oxantin) added, and any colour change noted. A 5 per cent. solution of hydrobromic acid is then added, drop by drop, until the maximum colour is developed, and finally water is added, also drop by drop, and any further change in colour noted.

(2) A crystal of tartaric acid smaller than a pin's head is gently warmed with a solution of 0·05 grm. of the phenol in the concentrated acid until fumes appear, and the colour noted.

(3) To obtain the greatest amount of differentiation 2·5 c.c. of a mixture of 1 c.c. of formalin and 99 c.c. of concentrated sulphuric acid are mixed with 2·5 c.c. of a solution of the phenol (0·01 to 0·05 grm.) in sulphuric acid. Water is added, drop by drop, and the colour change noted. If a dark precipitate is obtained, it is best to add the mixture slowly to 6 c.c. of alcohol.

The three following tests are more suited to mixtures of phenol with other substances.

(4) An extract of the sample, free from alcohol and resin, in 10 c.c. of 0·5 – 0·75 *N* hydrochloric acid, diluted, if possible, till almost colourless, is precipitated with 5 drops of formalin at the boiling point, cooled, re-heated and cooled, and the precipitate filtered off from the hot solution.

(5) Dihydroxyacetone may replace formalin in acid solutions not weaker than 7·5 *N*, but this reagent is less suitable for separation purposes. A better colour is often obtained by the addition of a few drops of hydrogen peroxide at the moment the colour change appears.

(6) Stain tests may be carried out with a deal shaving dipped in the solution, dried, dipped in hydrochloric acid, and gently warmed. Alternatively, a solution of 0·01 grm. of the phenol in 1 c.c. of alcohol, with 1 drop of formalin or dihydroxyacetone solution, is allowed to fall on two filter papers, one above the other, on a warm tile, and 5 drops of hydrochloric acid added while the papers are still moist. The colour changes are noted when the papers are almost dry, and again after the addition of the drop of hydrogen peroxide and 2 drops more of acid, and finally a little ammonia. The results of these tests will be found tabulated for a number of phenols.

Determination of Eugenol in Oil of Cloves. Van Ork (*Chem. Zentrlbl.*, 1925, ii., 1393) recommends a titration method for the determination of eugenol in oil of cloves, the advantages claimed being that the determination can be completed in half an hour, and that any alcohols that may be present do not interfere with the results.

One grm. or thereabouts of the oil is introduced into a glass-stoppered flask, and 25 c.c. of a 3 per cent. solution of caustic soda in water are

added. The mixture is well shaken and 22 grms. of sodium bromide are added. The whole is well shaken repeatedly and allowed to stand for half an hour. The mixture is filtered, and 20 c.c. of the filtrate are titrated with N/2 hydrochloric acid, methyl orange being used as indicator. Towards the end of the titration ether is added to prevent a turbidity due to non phenolic bodies. A correction is applied for the increase in volume due to the addition of the sodium bromide, which appears to be employed as a "salting out" salt, by a blank experiment, the increase in volume by the addition of the salt being measured.

This process has not been accepted as a substitute for the usual alkaline absorption process.

OXIDES, ETC.

The Determination of Cineol.—The determination of cineol in eucalyptus and similar oils has always been a matter of considerable difficulty, and it has been universally recognised that there is no really accurate method at present known. The method at present official in the British Pharmacopœia, depending on the formation of eucalyptol phosphate, is so empirical, and varies so much according to the variations in details which cannot be set out, that continual disagreement between different analysts is always to be expected. The same is true of every other published method until recently. It is also to be observed that the method here described is open to criticism as being based on somewhat empirical principles, but it appears certain that it gives more concordant, and probably more accurate, results than any other process yet recorded.

This method was originated by Cocking (*Year Book of Pharmacy*, 1920, 395; *Pharm. J.*, June 25th, 1927). It has been carefully investigated by the Essential Oil Sub-Committee to the Standing Committee on Uniformity of Analytical Methods, and their report is as follows (*Analyst*, 1927, **52**, 276).

The ortho-cresol method, as it may be called, consists in determining the freezing point of a mixture of 3 grms. of the oil, previously dried over granular calcium chloride, with 2·4 grms. of ortho-cresol. The resulting "cresuleol" melts at a temperature varying with the amount of impurities present. It may well be

objected that this melting point will depend, not only on the quantity, but on the nature of the other bodies present. Whilst this is true, the character of the bodies other than cineol present in natural essential oils is sufficiently constant to render the melting point a substantially accurate indication of the amount of cineol present.

The test is carried out in a stout-walled test-tube, about 15 mm. in diameter and 80 mm. in length, fitted with a wire loop for suspending it from the stirrup of a balance. Three grms. of the oil and 2·1 grms. of melted ortho-cresol are weighed into the tube by means of fine pipettes, so that 1 drop will allow accurate weighing to within 1 per cent. An accurate thermometer, graduated in is inserted, the mixture well stirred to induce crystallisation, and the highest reading of the thermometer noted. The tube is then warmed until the contents are melted, and is then inserted through a bored cork into a wide-mouthed bottle to act as an air jacket, and allowed to cool slowly until crystallisation commences or the thermometer has fallen to the previously noted temperature. It is then stirred vigorously with the thermometer, the latter being rubbed on the side of the test-tube with an up-and-down motion to induce rapid crystallisation, the stirring and rubbing being continued as long as the temperature rises. *The highest point is taken as the freezing point.* The mixture should be remelted and the test repeated until two concordant results are obtained, as the first temperature noted is always lower than the true freezing point. Corrections for emergent column are unnecessary, as any error thereby introduced is so small as to be negligible. Differences in the size of the thermometer bulb do not cause any variation in the freezing points recorded.

With oils of low cineol content, it may be necessary to introduce a minute crystal of the ortho-cresol-cineol addition compound,* to start crystallisation.

This method is satisfactory for oils containing 50 per cent. and upwards of cineol. Oils containing less than 50 per cent. may be mixed with an equal weight of pure cineole or a high-content oil before carrying out the test. A better way, however, is to perform the test in the usual manner first, and then, if the mixed liquids do not crystallise, add an equal weight (5·1 grms.) of pure recrystallised ortho-cresol cineol compound,* warm until liquefied, determine the freezing point as before, and make the necessary corrections.

* The pure ortho-cresol-cineol compound may be prepared from a high percentage oil by mixing with ortho-cresol, cooling, draining and pressing the crystalline magma, and recrystallising from a small quantity of petroleum spirit. The freezing point of this compound should not be below 55·2° C.

The following table of freezing points gives the percentages of cineol in oil :

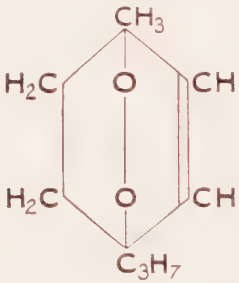
Freezing point of 3 grms. oil + 2·1 grms. o-Cresol. °C.	Cineol by weight. Per cent	Freezing point. °C.	Cineol by weight. Per cent.
24	45·6	40·4	67·5
25	46·9	41	68·65
26	48·2	42	70·5
27	49·5	43	72·35
27·4	50·0	44	74·2
28	50·8	45	76·1
29	52·1	45·2	76·5
30	53·4	46	78·0
31	54·7	47	80·0
32	56·0	48	82·1
33	57·3	48·4	83·0
34	58·6	49	84·2
35	59·9	50	86·3
36	61·2	51	88·8
37	62·5	52	91·3
38	63·8	53	93·8
39	65·25	54	96·3
39·8	66·5	54·6	98·0
40	66·8	55	99·3
—	—	55·2	100·0

It is important that the ortho-cresol should be pure and dry, and, as it is hygroscopic, it should be stored in small well-stoppered bottles. Penfold and Morrison (*Perf. and Ess. Oil Rec.*, 1928, 468) agree that the method is an excellent one and should be adopted by the British Pharmacopœia authorities. They point out, however, that abnormal amounts of cineol were found in oils of the *E. australiana* and *E. dives* types, due to the presence of *a*-terpineol. Although this variation may not often occur in oils of the usual medicinal standard, Penfold and Morrison consider it is too great to be overlooked, and suggest that the determination should be made on the portion distilling below 190°. This method has also been examined and reported upon very favourably by Berry (*Australasian J. of Pharm.*, March, 1929).

The Determination of Ascaridole in Chenopodium Oil. Ascaridole, $C_6H_6O_2(CH_3)(C_3H_7)$, is the principal constituent of American wormseed oil, in which it is present to the extent of 60 to 75 per cent. Nelson (*J. Amer. Pharm. Assn.*, 1921, **10**, 836)

suggested the following method for its determination, which is a matter of considerable importance, since it is the only constituent of the oil having anthelmintic action. Ten c.c. of the oil are placed in an ordinary cassia oil flask, and to this are added 75 c.c. of a solution of 60 per cent. acetic acid. The mixture is then thoroughly shaken and the unabsorbed oil raised to the graduated neck by the addition of more acetic acid. The absorption of the ascaridole by acetic acid is not, however, sufficiently quantitative, and the method is hardly accurate enough to be of much value.

Paget (*Analyst*, 1926, **51**, 170) has examined the subject minutely, and finds that ascaridole, which was shown by Wallach to be reducible, is best reduced by titanous chloride or sulphate for the purposes of quantitative determination. The structure of ascaridole is believed to be as follows :



Under no conditions could Paget obtain reduction results which corresponded to any theoretical alteration in this formula. At the same time, he has evolved a process which rests on the empirical basis that 1 grm. of ascaridole is reduced by 1·2770 grm. of titanous chloride. This figure is taken as the mean of results varying from 1·242 to 1·304 grms. The process is as follows :

Titanous chloride solution was prepared from the commercial 15 per cent. solution, 66 c.c. of which is diluted to 2,250 c.c., or standardised so as to contain 0·003856 grm. of titanous chloride per c.c. One grm. of the oil is diluted with 96 per cent. alcohol to 100 c.c., and 10 c.c. of this is transferred to a flask through which a current of carbon dioxide

passes, and 50 c.c. of titanous chloride solution are added. The flask is then closed with a Bunsen valve, and its contents heated almost to boiling point for two minutes. If the pale violet colour of the titanous chloride disappears, more is added to ensure an excess. One c.c. of a 5 per cent. of potassium thiocyanate solution is added, and the solution titrated with a standard solution of iron alum until a permanent faint red colour appears.

An important paper has been published (*Analyst*, 1930, **55**, 180) on this subject by Cocking and Hymas, who give the following summary and details of their work :—

The following methods have been used for the determination of ascaridole in chenopodium oil :—

(1) Separation of ascaridole by repeated fractionation *in vacuo*.

(2) Solution of the ascaridole by shaking the oil with 60 per cent. acetic acid and subsequently measuring the unabsorbed portion of the oil, the ascaridole being taken by difference. This method was first suggested by Nelson (*J. Amer. Pharm. Assoc.*, 1921, **10**, 836), and has been adopted by the United States Pharmacopœia. It suffers from the disadvantage that adulterants such as cineole are also soluble in 60 per cent. acetic acid.

(3) Reduction of the ascaridole by means of excess of titanous chloride with subsequent titration of the excess with ferric alum. This method was introduced by Paget (*Analyst*, 1926, **51**, 170 ; *vide supra*), and while it is much superior to the other methods, inasmuch as it is not affected by the usual adulterants, it suffers from the inconvenience that the whole determination must be carried out in an atmosphere of carbon dioxide.

In the hope of finding a more convenient method than Paget's titanous chloride reduction, experiments were tried with a number of reducing agents. Stannous chloride and ferrous thiocyanate were found to react readily with the oil, but the reagents themselves are rapidly oxidised by atmospheric oxygen, and the methods therefore suffer from the same objection as the titanous chloride process.

Eventually the reducing effect of potassium iodide in acid solution was tried and found to result in an immediate liberation

of iodine. As this reagent is relatively stable towards atmospheric oxygen, the following series of experiments was made :—

An approximately 5 per cent. (w v) solution of chenopodium oil in 90 per cent. acetic acid was prepared, and 5 c.c. of this solution, referred to as "Solution A," measured from a burette, was taken for each test.

(1) 5 c.c of solution A were mixed with
15 c.c. of glacial acetic acid and
10 c.c. of approx. $N/1$ potassium iodide solution.
An immediate liberation of iodine occurred, which increased on standing ; after remaining at laboratory temperature for two and half hours, it was titrated with $N/10$ thiosulphate,
required 23·5 c.c. per grm. of oil.

(2) As (1) but the solution was alternately warmed in a steam-bath and titrated to a permanent end-point ; time about twenty minutes,
required 31·5 c.c. per grm. of oil.

(3) As (1) but with the addition of 5 c.c. of hydrochloric acid,
required 31·7 c.c. per grm. of oil.

(4) A stronger solution of potassium iodide was now tried.
5 c.c of solution A were mixed with
10 c.c. of glacial acetic acid and
3 c.c. of approx. $5N$ potassium iodide solution.
This was alternately warmed in the steam-bath and titrated as before,
required 32·2 c.c. per grm. of oil.
To the solution were now added 10 c.c. of hydrochloric acid, when a further liberation of iodine took place, and titration with thiosulphate corresponded to a further 22·0 c.c. per grm. of oil.
or a total of 54·2 c.c. per grm. of oil.
At the completion of the titration, the liquid had a slight yellow colour which was not removed by more thiosulphate, in contradistinction to the end-point when acetic acid only was used, when the liquid was colourless.

(5) 5 c.c. of solution A were mixed with
3 c.c. of approx. $5N$ potassium iodide solution and
5 c.c. of conc. hydrochloric acid.
A copious liberation of iodine occurred immediately, and this was titrated at once ;
required 72·3 c.c. per grm. of oil.

(6) As (5) but allowed to stand at laboratory temperature for one and a half hours before titrating ;
required 64·0 c.c. per grm. of oil.
In this case after titrating there was a dark oily scum, and it

is evident that, on standing, some of the iodine was absorbed by the oil.

(7) In this experiment the same quantities were used, but the potassium iodide and hydrochloric acid were mixed together before adding solution A, and then titrated immediately ;
required 70·5 c.c. per grm. of oil.

(8) The acid was increased to 10 c.c., that is :—
3 c.c. of approx. 5*N* potassium iodide solution were mixed with
10 c.c. of conc. hydrochloric acid and then
5 c.c. of solution A were added, and the
mixture titrated immediately ;
required 78·1 c.c. per grm. of oil.

(9) The same as (8) but with the addition of 5 c.c. of carbon tetrachloride to the acid mixture before adding solution A ;
required 80·8 c.c. per grm. of oil.

(10) As (9) but with 5 c.c. of benzene instead of carbon tetrachloride ;
required 61·7 c.c. per grm. of oil.

The layer of benzene appeared to have the effect of delaying the reaction, and it was found that the highest results were obtained when the mixing was as rapid as possible.

In these last four experiments it was noticed that the mixture became appreciably warm on the addition of solution A, so the effect of cooling the acid mixture before adding the solution of the oil, was tried.

(11) 3 c.c. of approx. 5*N* potassium iodide solution were mixed with
10 c.c. of conc. hydrochloric acid and
5 c.c. of carbon tetrachloride and
cooled in freezing mixture, and then
5 c.c. of solution A were added and the mixture titrated immediately ;
required 94·5 c.c. per grm. of oil.

(12) As (11) but without the carbon tetrachloride ;
required 97·5 c.c. per grm. of oil.

These last conditions appeared to give the best results and a number of experiments were carried out by three different observers, with the following results :—

97·9, 98·2, 95·8, 98·9, 98·9, 98·2 c.c. per grm. of oil.

When the reaction mixtures were allowed to stand a long time before titrating, low results were invariably obtained ; the appearance of the liquid at the end of the titration was also different. After a titration carried out under the best conditions, the liquid appeared as a uniform colourless white very turbid liquid ; if the titration was delayed, or the conditions not adhered

to, then the final liquid was yellowish, turbid and with a dark oily scum, and the lower the result, the darker the scum. Excess of thiosulphate did not remove the yellow colour, and the titration was very liable to be overrun.

At this point, it was decided to test the method on pure ascaridole itself, and Dr. T.A. Henry, of the Wellcome Chemical Research Laboratories, kindly supplied some ascaridole of 96 per cent. purity, as tested by the titanous chloride method. With this ascaridole, the method as outlined above was tried, and a result was obtained, which, on the assumption that ascaridol liberated 2 atoms of iodine per molecule, corresponded with 110 per cent. This was not entirely unexpected, as the figures for the chenopodium oil itself indicated a percentage higher than that of a normal oil.

Further experiments were carried out in an attempt to discover the conditions whereby a theoretical figure would result.

On the assumption that ascaridole reacts like a normal peroxide and liberates two atoms of iodine per molecule, we found that, by altering the quantities of the reagent, it was possible to get results approximating very closely to the theoretical figure, but unfortunately very slight variations of the conditions were sufficient to vitiate the tests and send the results either up or down, mostly up.

The experiments seem to indicate that there are three separate reactions taking place: the first being the normal peroxide liberation of two atoms of iodine per molecule from acidified potassium iodide; the second reaction is a further liberation of iodine, the mechanism of which cannot at present be explained; while the third is the re-absorption of the iodine after its liberation; this last takes place when the reaction mixture is diluted.

As the conditions under which the several reactions would balance and give concordant results which could be expressed by a simple equation, could not be found, attempts were made to discover the conditions that would allow a reasonable amount of latitude in working, and then to employ a factor standardised on pure ascaridole.

The reaction between the last-mentioned reagent (Expt., No.

12) and the oil was extremely rapid, being completed in less than thirty seconds. The next step was to delay this reaction by the addition of glacial acetic acid, while at the same time reducing the amount of hydrochloric acid.

The experiments showed that, unless an excess of hydrochloric acid over the equivalent of the potassium iodide was present, the reaction did not proceed very far. Eventually it was found that identical results could be obtained when 3 c.c., 4 c.c., or 5 c.c. of the hydrochloric acid were used, but the results with 5 c.c. were more uniform when tested by other observers. The method finally adopted is as follows :—

About 2·5 grms. of the oil, accurately weighed are dissolved in sufficient 90 per cent. acetic acid to produce 50 c.c., and this solution is placed in a narrow burette. Three c.c. of potassium iodide solution, approximately 5 N (83 per cent. w/v) and 5 c.c. of concentrated hydrochloric acid (B.P. strength) are placed in a stoppered tube of about 60 c.c. capacity, and 10 c.c. of glacial acetic acid are added. The tube is cooled in a freezing mixture to about −3° (permissible limits 0° to −3°), then removed, and 5 c.c. of the acetic acid solution of the oil run in from a burette, being mixed with the reagent as quickly as possible and due allowance being made for the draining down of the burette. The tube is stoppered and allowed to stand in a cool place for five minutes. The reaction appears to be complete in two minutes, but five minutes give slightly more concordant results; and, provided the final temperature of the reaction mixture does not exceed 10°, ten minutes may safely be allowed. The contents of the tube are next titrated directly with N/10 thiosulphate solution, and if the conditions have been adhered to a sharp end-point will be obtained, and the final titration liquid will be quite colourless and very turbid, the turbidity being due to fine oil globules in suspension.

A blank test on the reagents is carried out at the same time and under the same conditions, except that the mixture is diluted with 20 c.c. of water before titrating and the reading subtracted from that obtained in the test. Each c.c. of N/10 thiosulphate solution is equivalent to 0·00665 grm. of ascaridole.

Seven determinations by the above method were carried out on the sample of 96 per cent. ascaridole, and the results showed a variation corresponding to an ascaridole equivalent per c.c. N/10 thiosulphate of 0·0660 to 0·0670 grm., with a mean result of 0·0665 grm.

In twenty-eight tests on a sample of chenopodium oil by the above method, the results showed a maximum divergence of 2 per cent., that is, an experimental error of ± 1 per cent.

It was found that, if the reaction mixture was diluted before titration, low results were invariably obtained and the end-points were bad. The following diluents were tried: water, iced water, brine, iced brine, 10 per cent. hydrochloric acid, solutions of sodium sulphate, ammonium sulphate, ammonium acetate, ammonium chloride, and potassium iodide. With the exception of potassium iodide solution, all gave low and very variable results: with potassium iodide solution most of the results were slightly low, but in several instances results identical with those given by undiluted mixtures were obtained. It is possible that dilution with potassium iodide solution might be successful, provided the exact conditions were worked out.

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CHAPTER IV

THE PROTEINS

By Dorothy Jordan Lloyd, M.A., D.Sc., F.I.C.

The Isolation of New Units—The Determination of Special Units—The Nature of the Linkages in the Protein Molecule—The Analysis of Proteins by means of Enzyme Action—The Size of the Unit Aggregate—Electrodialysis as a Method of Protein Purification—Physical Methods in the Determination of the Size of the Primary Particle. *Biological Analysis of Proteins* (by Harriette Chick, D.Sc.): Relative Value of Proteins for Rearing Young Animals to Maturity—Relative Value of Proteins for Maintaining Nitrogenous Equilibrium.

I. THE ISOLATION OF NEW UNITS

At the beginning of the nineteenth century, the constitution of the proteins was one of the great unsolved problems of organic chemistry. Built up of only six elements, C, H, O, N, S and sometimes P, the proteins yet formed a well-defined group of substances, apparently almost unlimited in number, since every animal or plant species examined contributed new members to the group. This multiplicity of the proteins as a class was not at first appreciated, and much of the early work in protein analysis aimed, unsuccessfully, at isolating an organic grouping of fixed chemical constitution and properties, which was to be regarded as the chemical basis of living matter. From Mulder's early papers (*Berzelius Jahresb.*, 1839, **18**, 534; *Id.*, 1840, **19**, 639), in which he put forward the theory that the proteins are compounds of sulphur and phosphorus with an organic radicle, which he called "protein," and to which he gave the formula $C_{19}H_{62}N_{19}O_{19}$, down to Kossel's more recent theory (*Ber.*, 1901, **34**, 3214), that all proteins are built round a central "protamine nucleus" of fixed constitution, consisting of arginine ($CNH_2NHNH.CH_2.CH_2.CH_2.CHNH_2.COOH$), the idea that proteins are

characterised by possessing a primary organic grouping common to the class as a whole, has continually appeared in the literature. Larmour (*Trans. Roy. Soc. Can.*, 1928, **22**, 349) and Cahn (*Ann. de Physiol. et de Physicochem. Biol.*, 1928, **4**, 667) have both brought forward evidence in support of Kossel's "protamine nucleus" theory, even as recently as within the last two years. The possibility that the proteins associated with cell life may be built round an organic grouping common to the class cannot, therefore, be regarded as finally disproved, although it seems at present more probable that the biological significance of many members of the group is based mainly on their physico-chemical properties (see Jordan Lloyd, *Biological Reviews*, 1928, **3**, 165). It is, however, a fact not to be ignored that in the reconstruction of the cell proteins that occurs during the formation of highly active biological tissues, such as the generative cells, the simple mono-amino acids (the significance of which in the protein is probably more physical than chemical) are largely eliminated, with a consequent increase in the percentage of the basic amino acids, arginine, histidine or lysine, each with its own strongly marked chemical individuality. Possibly in this way maximum chemical activity is secured in the minimum amount of space.

The history of the early attempts at protein analysis has been reviewed by Vickery and Osborne (*Physiological Reviews*, 1928, **8**, 393) in a paper which throws much light on the aims of the earlier workers. It is not proposed, therefore, to deal here with methods which have long since been abandoned. The methods that gradually became paramount were built up from the work of Drechsel, Kossel, E. Fischer, Hofmeister and many others. Fischer's method of analysis, which became a standard practice for many years, consisted in a hydrolysis of the protein with strong acid, separation of the products of hydrolysis, after neutralisation, into a basic and non-basic fraction by precipitation with phosphotungstic acid, and further separation of the non-basic units by esterification and fractional crystallisation. It gradually became clear, after the work of Fischer and Hofmeister, that the common constitutional characteristic of the proteins is that they are condensation products of amino acids, the condensation

occurring mainly by means of a peptide linkage involving the carboxylic grouping of one unit with the amino grouping of its neighbour. The character of any individual protein is determined by the nature and amount of its constituent amino acids and by the arrangement of these in the molecule. Twenty-six amino acids have now been isolated, together with two imino acids, proline and hydroxyproline, which are frequently present in large amounts. Fischer's esterification method for the separation of the non-basic units has, in recent years, been greatly improved by Dakin (*Biochem. J.*, 1918, **12**, 290), who introduced extraction of the neutralised products of hydrolysis with butyl alcohol before the removal of the basic units with phosphotungstic acid and the separation of the non-basic units by esterification. Kingston and Schryver (*Biochem. J.*, 1924, **18**, 1070) have introduced a new method of separation, based on the fact that amino acids in solution in aqueous alcohol form crystalline carbamates in the presence of carbon dioxide and barium hydroxide. A detailed summary of these three general methods of separation, with references to the original papers, are given in Jordan Lloyd's *Chemistry of the Proteins*, together with accounts of other methods which have been used particularly for the separation of special units. The striking advance to which the use of Dakin's and Schryver's methods has led, has been the separation of a number of new hydroxy amino acids—in fact, it appears possible that every known amino acid may also occur naturally in the oxidised as well as the normal form.

In view of the large number of hydroxy amino acids recently isolated by Schryver and his colleagues, a detailed description of the use of the carbamate method for the analysis of gelatin is given on p. 128.

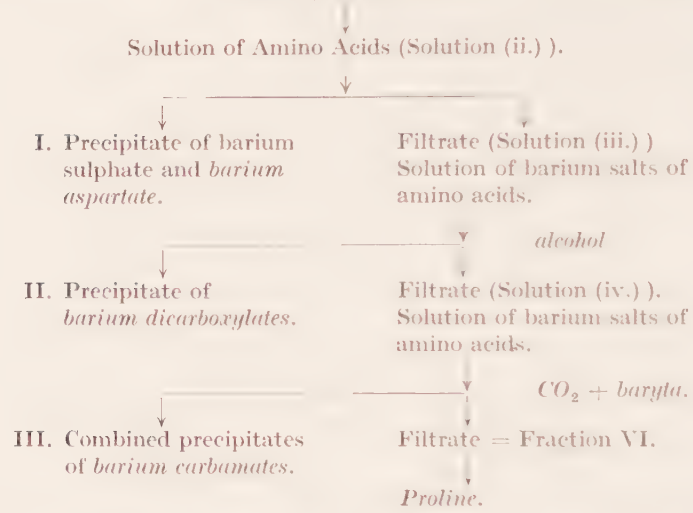
Solution (i.).—Nine hundred grms. of gelatin in 4,500 c.c. of water. Add H_2SO_4 up to a concentration of 25 per cent., and boil for thirty hours.

Solution (ii.). Dilute to 5,400 c.c. and add $Ba(OH)_2$ till just distinctly acid to Congo red. Filter off the precipitate of $BaSO_4$.

Solution (iii.). Titrate any free COOH groups with formalin, and add the calculated amount of $Ba(OH)_2$ to complete the conversion of all amino acids into Ba salts. Filter off the precipitate of $BaSO_4$ and barium aspartate (I.). Concentrate to 3 litres, add 6 litres of 95 per

cent. alcohol and stand for two days. Filter off the crystalline precipitate of barium dicarboxylates (II.).

Solution (iv.). *Process of Carbamation.* Cool with ice, add phenolphthalein (as indicator) and fresh, finely powdered or recrystallised $Ba(OH)_2$ to saturation. Pass CO_2 gas, with stirring, till phenolphthalein


Gelatin (Solution (i.)).
Solution of Amino Acids (Solution (ii.)).
I. Precipitate of barium sulphate and *barium aspartate*.
Filtrate (Solution (iii.)) Solution of barium salts of amino acids.
alcohol
II. Precipitate of *barium dicarboxylates*.
Filtrate (Solution (iv.)). Solution of barium salts of amino acids.
CO_2 + *baryta*.
III. Combined precipitates of *barium carbamates*.
Filtrate = Fraction VI.
Proline.

is discoloured. Add more baryta to saturation and more CO_2 for two hours and repeat twice. Filter and repeat carbamation twice more.

Add baryta to precipitate excess of CO_2, filter off $BaCO_3$ and evaporate under reduced pressure to 1 litre. Add 2 volumes of 95 per cent alcohol, cool with ice and carbamate three times. Add the precipitates to the other fractional precipitates of the carbamation process (III.).

Wash precipitate III. with 2 volumes of 95 per cent. alcohol. Dry with alcohol and ether. Add the aqueous alcoholic washings to the combined filtrates of the carbamation process.

Fraction VI consists of the combined filtrates from the carbamate precipitates. Concentrate *in vacuo* to a syrup. Dissolve in water and hydrolyse in 25 per cent. H_2SO_4 for twelve hours. Remove the acid with baryta, filter, concentrate to a syrup as before and throw into absolute alcohol. Repeat the hydrolysis until the product is completely soluble in absolute alcohol. *Proline* can be crystallised out as a cupric salt.

Precipitate III. –Extract in succession with 5, 2·5, 1·5 and 1 litre of ice-cold water. Filter. Insoluble residue = Precipitate IV.

Solution (v.). Boil and filter off $BaCO_3$ and dilute to a 2 per cent. solution of the amino acids. Precipitate di-amino acids by phosphotungstic and sulphuric acids (Precipitate V.).

Solution (vi.). Remove excess of phosphotungstic and sulphuric acids with $Ba(OH)_2$. Concentrate *in vacuo* till leucine begins to separate. Filter off leucine, concentrate to small volume and throw into absolute alcohol. Precipitate of mono-amino acids. Filter.

Solution (vii.). Evaporate down and take up with absolute alcohol. Filter. Repeat until a product is obtained completely soluble in absolute alcohol, *i.e.*, *proline*.

Precipitate of barium carbamates (III.).

↓ *extraction with ice-cold water.*

IV. Insoluble barium carbamates.

Filtrate (Solution (v.)).
Solution of barium carbamates.

heating.

Glycine.

Solution of amino acids.

V. Precipitate of *di-amino acids.*

Filtrate (Solution (vi.)).
Solution of mono-amino acids.

(*a*) Crystalline precipitate.
Leucine.

(*c*) Precipitate containing
alanine
phenylalanine
hydroxyproline
leucine (remains of)
serine

(*b*) Filtrate (Solution (vii.)).

Proline.

Precipitate I. Boil with H_2SO_4 and precipitate excess H_2SO_4 with $Ba(OH)_2$. Filter. Concentrate filtrate to a syrup and throw into absolute alcohol. Aspartic acid crystallises out.

Precipitate IV. Suspend the *insoluble carbamates* in hot water and blow steam for two hours. Filter off the $BaCO_3$, concentrate *glycine* crystallises out.

It would be out of place in a report which deals only with recent work to give in detail an account of each of the known

amino acids. The names and formulae of these, with the date of their first recognition, will be found in Jordan Lloyd's *Chemistry of the Proteins*, and the method of isolating each member and an account of its properties will be found in Plimmer's monograph on *The Chemical Constitution of the Proteins* and in Oppenheimer's *Handbuch der Biochemie*, 1924, Vol. I., 2nd Ed. A certain number of new units have been isolated as the result of the use of modern methods, and these are listed below :

Hydroxyglutaminic Acid.—Isolated in 1914 by Dakin from casein by means of the butyl alcohol extraction method (*Biochem. J.*, 1918, **12**, 290).

Hydroxylysine, or ϵ-α-amino-β-hydroxy-caproic acid.—Isolated in 1925 by Buston, Schryver and Mukerjee from isinglass by means of the carbamate method. The barium carbamate of hydroxylysine differs from that of most of the other bases by its insolubility in water (*Proc. Roy. Soc.*, B, 1925, **98**, 58). Hydroxylysine has been found in a number of vegetable proteins and in gelatin. It is absent from casein and ovalbumin.

Hydroxyaminobutyric Acid and Hydroxyvaline.—Isolated in 1926 by Schryver and Buston from the hydrolytic products of the protein from oats (*Proc. Roy. Soc.*, B, 1926, **99**, 476). Gortner and Hofmann (*J. Amer. Chem. Soc.*, 1925, **47**, 580) believe that they have isolated hydroxyaminobutyric acid from teozein, and it has also been isolated by Rimington from casein (*Biochem. J.*, 1927, **21**, 1179).

Schryver had turned his attention to vegetable proteins, believing that these products were more likely to yield new units than animal proteins. In view of his successes, which have opened up a new field of investigation, his method of preparing the alkali-soluble oat protein is given here.

" Fine oatmeal, in several batches each of 300 grms., was made into a thin paste with water, and stirred into 2,000 c.c. of 0·4 per cent. caustic soda ; the mixture was allowed to stand for about two hours after which period the starch in the oatmeal had become gelatinised. Hydrochloric acid was carefully added to the mixture till the pH was restored to about 7, as indicated by bromthymol blue. A small amount of taka-diastase was well stirred in, together with a little toluene, and the whole kept for twenty-four hours at 37° in an incubator.

By this time, the product had attained the consistence of a thin liquid, the starch having been hydrolysed. A little caustic soda was added to dissolve any separated protein, and the liquid filtered, first through muslin to remove coarse material, and then through a Sharples centrifuge. A pale brownish, opalescent filtrate was thus obtained, from which a copious white precipitate was produced on the addition of hydrochloric acid. This precipitate of crude protein was removed on the Sharples centrifuge, washed thoroughly with water, and then extracted for some hours with boiling 70 per cent. alcohol, to remove gliadin-like proteins. After this it was again washed with water and extracted with 10 per cent. sodium chloride to remove nucleic acid (method of Clarke and Schryver).

"The protein was finally purified by dissolving in 0·2 per cent. caustic soda, filtering through paper-pulp and reprecipitating by dilute acid. It was dried by washing with graded strengths of alcohol, and finally with ether. In all, 18 kilos of oatmeal yielded 730 grms. of dry protein, containing 14·4 per cent. of nitrogen."

The oat protein was hydrolysed by boiling with concentrated sulphuric acid, and the various fractions were separated by the carbamate method. The non-basic fraction from the ice-cold water extract of the barium carbamates was separated by the conversion of the amino acids into zinc salts. A fraction of these, soluble in water and alcohol, was further separated by the formation of the copper salts. A salt insoluble in methyl alcohol yielded hydroxyaminobutyric acid, while the fraction soluble in methyl alcohol gave hydroxyvaline.

Protoctine.—Isolated in 1926 by Schryver and Buston from the basic fraction of the barium carbamates from the hydrolysis products of oat and castor-bean proteins (*Proc. Roy. Soc.*, B, 1926, **100**, 360). Protoctine is a base soluble in absolute alcohol. It has a formula, $C_{11}H_{21}O_2N_3$. The constitution is still unknown, but it is said to have one amino, one carboxylic and one hydroxyl group. Its basic character and its colour reactions with diazobenzene sulphonic acid suggest a similarity to histidine.

***dl*-Lysine.**—The optically inactive form of lysine has been found by Schryver and Buston (*Proc. Roy. Soc.*, B, 1928, **101**, 519), to the extent of nearly 1 per cent., in the hydrolysis products of gelatin which has been allowed to stand for twenty-four hours in contact with concentrated, strong acid at room temperature before hydrolysing at boiling temperature. It comes down in the arginine fraction, from which it is separated by the removal of arginine as a salt of flavic acid (Kossel and Standt, *Z. physiol.*

(*Chem.*, 1926, **156**, 270). The *dl*-lysine is crystallised as the picrate.

Schryver and Buston claimed that *dl*-lysine was a naturally occurring component of gelatin, basing their belief on the observation that the basic fraction of gelatin is not so great if hydrolysis with boiling strong acid takes place immediately as it is if the gelatin stands in preliminary contact with cold acid for twenty-four hours. This is ascribed to the release, under the latter conditions, of a new amino acid, inactive lysine, which is presumably destroyed under the former. The percentage of the ordinary active form of lysine obtained under the two conditions is about the same. Daft, however (*C. R. Lab. Carlsberg*, 1929, **17**, No. 12), has been unable to confirm this work.

The use of alkalis or digestive enzymes as agents for protein hydrolysis occurs in the earliest stages in the history of protein analysis. Later, hydrolysis by boiling strong acid became a standard practice, although it was soon recognised that in an analysis of a protein a preliminary boiling in a concentrated solution of hydrochloric or sulphuric acid was a treatment liable to destroy a certain number of the less stable units. For this reason, less drastic methods were again adopted by certain workers. Hopkins and Cole, in 1901 (*J. Physiol.*, 1901, **27**, 418), achieved success by isolating tryptophane from a tryptic digest of casein. This acid is always completely destroyed in the customary hydrolysis with strong acid. Homer (*J. Biol. Chem.*, 1915, **22**, 369) and Onslow (*Biochem. J.*, 1921, **15**, 392 ; *id.*, 1924, **18**, 63) have shown also that tryptophane is released intact from proteins by hydrolysis with weak baryta solution. The adoption of milder methods of hydrolysis has recently led to the discovery of one more amino acid (methionine) and to the establishment of the fact that a disaccharide (glucosamine-mannose) may also occur as a unit in the intact protein. It has also led to the separation of a number of peptides from the protein molecule. There is no doubt that in the future the use of enzymes for protein hydrolysis will throw much light on the structural pattern in which the amino acids are arranged.

Methionine, or γ-methyl-thiol-α-aminobutyric acid. Isolated in 1923 by Mueller from a bacterial digest of casein (*J. Biol. Chem.*, 1923, **56**, 156), and by Odake from a yeast autolysate (*Biochem. Z.*, 1925, **16**, 446). Barger and Coyne, in 1928 (*Biochem. J.*, 1928, **22**, 1417), separated it from a tryptic digest of casein in the precipitate obtained on the addition of Hopkin's reagent (10 per cent. mercuric sulphate in 5 per cent. sulphuric acid). This precipitate can be decomposed by passing hydrogen sulphide through a suspension in water; the mercury remains as an insoluble sulphide, and the amino acids in the solution are separated by esterification and distillation. Barger and Coyne established the constitution of γ-methyl-thiol-α-aminobutyric acid and proposed the name of "methionine."

Glucosamine-mannose. Isolated in 1927 by Frankel and Jellinek (*Biochem. Z.*, 1927, **185**, 392) from the albumins of egg white and of egg yolk, and in 1929 by Rimington (*Biochem. J.*, 1929, **23**, 430) from the albumin and globulin of serum. The method employed is hydrolysis with a solution of baryta (about 10 per cent. concentration) and precipitation of the free carbohydrate with lead acetate and ammonia. The acetate is added first, any precipitate formed is filtered off, and ammonia is then added to the filtrate until the precipitation of the disaccharide is complete. The constitution of the disaccharide was established by Rimington.

II. THE DETERMINATION OF SPECIAL UNITS

In general, the amount of any amino acid which is combined in a protein molecule is still determined by obtaining it as a crystalline compound and weighing directly. The final separation of special units is frequently effected by crystallisation as salts of heavy metals or as other crystalline derivatives. References to a number of these methods will be found in the text-books already quoted. The well-known method of analysis by nitrogen distribution, first worked out by Haussmann (*Z. physiol. Chem.*, 1899, **27**, 95), and brought to a high degree of efficiency by Van Slyke (*J. Biol. Chem.*, 1911, **9**, 185), also gives fairly accurate determina-

tions of the basic units. In recent years, however, two new general methods for the determination of amino acids have been developed in considerable variety. The first of these is the determination of special units by means of colour reactions, and the second is the determination by selective enzyme action. Tyrosine, cystine, tryptophane have all been determined by colorimetric methods, and arginine by the decomposition with the enzyme arginase. Tyrosine and cystine have also been estimated by the absorption of bromine and iodine, respectively. Histidine can be estimated in the free state by the method of Koessler and Hanke (*J. Biol. Chem.*, 1919, **39**, 497), but the reaction cannot be used in the presence of proteins and possibly of protein hydrolysis mixtures. It will be noticed that the simple mono-amino mono-carboxylic acids of the aliphatic series remain, as ever, the most difficult to identify and determine.

The Colorimetric Determination of Tyrosine.—Folin and Ciocalteu (*J. Biol. Chem.*, 1927, **73**, 627) describe a method of estimating tyrosine, which they have devised to replace the earlier method of Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 421). Tryptophane must not be present. The method is as follows:

"By means of a long slender test-tube, transfer into a *new*, clean, *dry* Kjeldahl flask [of Pyrex glass] (250 c.c.) about 1 grm. of thoroughly dried protein material. The exact weight is obtained by weighing the tube before and after the transfer. Then introduce into the flask 2 c.c. of butyl alcohol (to prevent foaming), a couple of short spirals made from silver wire or silver foil (to prevent bumping), and, finally, 4 grms. of sodium hydroxide in the form of 20 per cent. solution. Insert into the neck of the flask a condenser made from a test-tube of such a size that it fits very loosely, yet rests firmly on the flask by means of its flange.

"The mixture should be boiled for 18 to 20 hours. For this boiling it is inadvisable to apply the flame directly to the bottom of the flasks. . . . Some form of improvised air bath should be used to secure an even application of heat. An iron crucible (diameter 7 cm.) is satisfactory. The boiling will continue perfectly smoothly if the silver coils are right, and provided that the condenser continues to function so that the butyl alcohol is not lost. It is not necessary to boil hard.

"At the end of the boiling period, remove the condenser, add 10 c.c. of water, and continue the boiling for ten minutes, to remove the

alcohol. Then remove the flame and, from a pipette, add immediately to the hot solution, drop by drop, but rather fast, 10 c.c. of 14*N* sulphuric acid (200 c.c. of concentrated H_2SO_4 diluted to 500 c.c.). It is quite essential that the first 10 c.c. of acid should be introduced into the alkaline solution while the latter is still quite hot. The addition of acid should, in fact, produce boiling. Unless the mixture becomes very hot, the silicic acid is apt to remain in colloidal solution, and the mixture will have to be discarded.

"The first 10 c.c. of acid are more than enough to neutralise the alkali in the flask. After the addition of 10 c.c. of acid shake thoroughly and cool. Then add 5 c.c. more of the 14*N* acid to produce the required acidity; rinse the contents into a 100 c.c. volumetric flask, dilute to volume, shake thoroughly, and filter. The filtration is slow, and the funnel should be covered with a watch-glass during the two-hour period required to get about 60 c.c. of filtrate.

"If more than 60 c.c. of filtrate is desired, it is best to start with 2 grms. of protein material. In that case, one should add 8 grms. of sodium hydroxide, and for neutralisation and acidification should use 20 c.c. and 10 c.c. of 14*N* sulphuric acid. The acidified digest is then diluted to 200 c.c. before filtering.

"The acidified protein hydrolysates should be kept in an ice box, or at least in the dark, unless all the desired determinations can be started rather promptly, for if the hydrolysates stand around exposed to light at room temperatures for many days they soon grow dark in colour, owing to decomposition of the tryptophane.

"Transfer to a 15 c.c. centrifuge tube 8 c.c. of the protein hydrolysate and add, drop by drop, from a height of about 3 cm., 4 c.c. of a 15 per cent. solution of mercuric sulphate in 6*N* sulphuric acid. No stirring is necessary. Let the mixture stand for two to three hours and centrifuge fairly hard for five minutes. Decant the supernatant liquid into a 100-c.c. volumetric flask, draining thoroughly and rinsing the edge of the centrifuge tube with about 2 c.c. of 0·1*N* sulphuric acid. The amount of tyrosine remaining with the tryptophane is perhaps a shade more than could be accounted for on the basis of the amount of mother liquor in the tube. To the sediment in the tube add 10 c.c. of a solution containing 1·5 per cent. mercuric sulphate in 2*N* sulphuric acid.

"Stir with a fine glass rod and let stand for ten minutes. Traces of precipitated tyrosine dissolve fairly easily in 2*N* acid and the added mercuric sulphate prevents the solution of any tryptophane. At the end of ten minutes, rinse the stirring rod with 2 c.c. of the same 1·5 per cent. mercuric sulphate solution. Centrifuge again and transfer this wash liquid to the flask containing the original mother liquor, not omitting to rinse the edge of the centrifuge tube.

"The standard is prepared as follows: Introduce into a second

100 c.c. volumetric flask 5 c.c. of a standard tyrosine solution in 2*N* sulphuric acid containing 1 mgrm. of tyrosine per c.c. Add 4 c.c. of the 1·5 per cent. mercuric sulphate solution and 12 c.c. of the 1·5 per cent. mercuric sulphate solution and about 7 c.c. of 0·1*N* sulphuric acid.

"To the standard and the unknown must further be added 6 c.c. of 7*N* sulphuric acid, for the total acidity in each flask should be approximately equivalent to 100 c.c. of normal acid. Heat the two flasks in boiling water for 15 minutes and then cool in cold water approximately to room temperature. Next add to each flask, with shaking, 1 c.c. of 2 per cent. sodium nitrite solution. Dilute to volume at once and make the colour comparison without undue delay, always, of course, first reading the standard against itself so as to adjust the colorimeter or the eye.

"If the standard is set at 20 mm., then 20, divided by the reading of the unknown, multiplied by 1·25 and by 5, gives the per cent. of tyrosine, provided that the hydrolysate represents exactly a 1 per cent. protein solution."

Colorimetric Determination of Tryptophane.—A method which has given satisfactory results in the hands of several workers is that of May and Rose (*J. Biol. Chem.*, 1922, **54**, 213). The basis of the method is that the protein undergoes gentle hydrolysis in the presence of *p*-dimethylaminobenzaldehyde, which combines with the tryptophane as it is released, producing a soluble derivative of a blue colour. The intensity of colour developed is matched in a colorimeter against that produced under parallel conditions by a standard solution of casein. May and Rose based their calculations of tryptophane content on the figure of 1·5 per cent. as the tryptophane content of casein. Breeze Jones, Gersdorff and Moeller (*J. Biol. Chem.*, 1924, **62**, 183) show that on the collected evidence of several workers 2·2 per cent. is a more reliable figure. A standard solution of tryptophane can also be used.

"The best conditions for the hydrolysis of the protein were obtained when 50 c.c. of concentrated C.P. HCl, 50 c.c. of water and 1 c.c. of the reagent (a 5 per cent. solution of *p*-dimethylaminobenzaldehyde in 10 per cent. sulphuric acid) were mixed. To this mixture weighed portions of protein (0·05 to 0·1 grm.) were added, and the mixture was incubated at 35° C. for twenty-four hours and then allowed to stand twenty-four hours or longer (or until the maximum depth of colour has developed) at room temperature. This procedure gave blue-coloured solutions when tryptophane-containing proteins were

hydrolysed. The solutions varied only in intensity of colour. When the hydrolysis was brought about by other means, there resulted solutions of less intense blue colour or solutions of a green or reddish shade.

"The colour produced was permanent for at least ten days and probably for a considerably longer time."

Other colorimetric methods for the estimation of tryptophane have been put forward by Furth and Lieben (*Biochem. Z.*, 1920, **109**, 124); by Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 433), and a modification of the last-named method by Folin and Ciocalteu (*J. Biol. Chem.*, 1927, **73**, 627); by Ragins (*J. Biol. Chem.*, 1928, **80**, 543).

The Colorimetric Determination of Cystine. The method of Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 421) has been found satisfactory by a number of workers. This method involves the use of the "uric acid reagent" of Folin and Denis (*J. Biol. Chem.*, 1912, **12**, 239). This reagent is prepared as follows:

"To 750 grms. of water add 100 grms. of the (sodium) tungstate and 80 c.c. of 85 per cent. phosphoric acid (H_3PO_4). Boil gently for two hours, using a reflux condenser to prevent undue concentration, cool and dilute to 1 litre. Two c.c. of this solution give the maximum colour obtainable with 1 mgrm. of uric acid."

This reagent should be added to the unknown fluid in an acid solution, but the mixture must be made alkaline before the colour will develop. Excess of alkali leads to fading of the colour; nitrates must be absent. The procedure for the determination of cystine is as follows:

"From 1 to 5 grms. of the dry protein and 25 c.c. of 20 per cent. sulphuric acid are transferred to a 300-c.c. Kjeldahl flask fitted with a Hopkins condenser. The mixture is boiled gently over a micro-burner for twelve hours, after which it is cooled, diluted to 100 c.c. and thoroughly mixed. From 1 to 10 c.c. of the solution are transferred to a 100 c.c. volumetric flask and to it are added first 20 c.c. of saturated sodium carbonate solution and then 10 c.c. of 20 per cent. sodium sulphite solution. The mixture is well shaken and set aside while the standard cystine solutions are prepared. The standard cystine solution is made to contain 5 per cent. sulphuric acid and 1 mgrm. of cystine per c.c. This solution keeps indefinitely.

"Two standards are prepared containing 1 and 3 c.c., respectively, of cystine solution or 1 and 3 mgrms. of cystine. Add to each 20 c.c. of saturated sodium carbonate solution and 10 c.c. of 20 per cent. sulphite and let stand for five minutes. Three c.c. of the uric acid reagent of Folin

and Denis are then added (with shaking) to each standard and to the unknown digestion mixture. The three flasks are allowed to stand for ten minutes; the contents are then diluted to the 100 c.c. mark and the colour comparison between the unknown and the standard nearest it in colour is made in the usual manner. There is no need for any undue hurry in the making of the colour comparison, for the slow fading which takes place is exactly the same in the unknown as in the case of the standard."

Another colorimetric method by means of the naphthoquinone cysteine reaction is described by Sullivan (*J. Biol. Chem.*, 1927, **73,** xiv.).

Cystine and cysteine can also be determined by the iodine absorption method of Okuda (*J. Chem. Soc. Abs.*, 1924, **126,** 792; *J. Biochem. Tokyo*, 1925, **5,** 201, 217).

The development of colorimetric methods for determining special units has led to great advances in the knowledge of the constitution of the different proteins. An entirely new line of attack has been opened recently by the realisation that enzymes can be made use of for the determination of special units.

The Use of Arginase for the Determination of Arginine.—This possibility was first suggested by Jansen for determining arginine (*Chem. Weekbl.*, 1917, **14,** 214; *Mahly's Jahresber.*, 1917, **47,** 202). The arginine was decomposed by arginase with the release of urea, and this was decomposed by urease with the release of ammonia, which was determined. The method has been developed by Hunter and Dauphinée (*J. Biol. Chem.*, 1925, **63,** xxxix); by Bonot and Cahn (*Bull. Soc. Biol. Chem.*, 1927, **9,** 1001), and by Narayana and Sreenivasaya (*Biochem. J.*, 1928, **22,** 1138). Urease is conveniently prepared from soya beans or Jack beans. Arginase is present in the tissues of mammals and fish (Hunter, *J. Physiol.*, 1924, **59,** xxxiv.), but is found in the most active form in mammalian liver. Hunter's methods of preparing urease and arginase are as follows:

Urease.—"A highly active solution of urease was prepared by extracting 10 grms. of Jack bean flour with 100 c.c. of 50 per cent. glycerol. The extract was filtered, adjusted with NaOH solution to a *p*H of exactly 6·8, and filtered or centrifuged again until absolutely clear."

Active urease in crystalline form has been prepared by Sumner and Hand (*J. Biol. Chem.*, 1928, **76**, 149; **78**, xxxiv.).

Arginase.—"A freshly excised rabbit's liver having been perfused with saline till practically free of blood, a portion of about 10 grms. was weighed to the nearest decigram, transferred to a mortar, treated with glycerol in the proportion of 1 c.c. for each gram of tissue, and ground until converted into a nearly homogeneous emulsion. This was transferred to a stoppered bottle, and set aside with frequent shakings for twenty-four hours. It was then filtered through cheese-cloth, shaken vigorously with about a quarter of its volume of toluene, and centrifuged. The resulting top layers of toluene, fat and tissue *débris* were siphoned off. The relatively clear and homogeneous extract remaining constituted a stock solution of arginase. For use in standardisation it was diluted tenfold with 50 per cent. glycerol."

Hunter and Dauphinee (*Proc. Roy. Soc.*, B. 1925, **97**, 209) describe an ingenious method of standardising their arginase preparation by means of the change of *p*H value (as shown by the usual colorimetric indicators) that occurs under standard conditions. The same method can be applied to the estimation of arginase.

"A stock solution of arginine was prepared by dissolving 4·203 grms. of arginine hydrochloride, containing 3·475 grms. of the base, in 500 c.c. of the 3·50 *M* phosphate buffer of *p*H 7·5, prepared by diluting to 500 c.c. a mixture of 150 c.c. of *M* 5 KH_2PO_4 and 123·6 c.c. of *M* 5 NaOH. When this solution is treated, as happens in a determination or in the experiment now under description, with one-fifth of its volume of a tissue extract, its composition, with respect to arginine and phosphate concentrations, becomes that defined as the standard; while through the combined influence of the arginine salt and the always slightly acid extract, the original *p*H of the buffer solution is reduced to about 7·4.

"Into each of a set of ten test-tubes were introduced (*a*) 5 c.c. of the stock arginine solution; (*b*) a measured amount, increasing serially by steps of 0·1 from 0·1 to 1·0 c.c. of the tenfold diluted extract; (*c*) sufficient water to make the total volume 6 c.c.; and (*d*) 5 drops of toluene. The tubes, having then been shaken and stoppered, were set in a thermostat at 20° for twenty-four hours.

"At the end of this interval, the contents of each tube were treated with 1 drop of 20 per cent. HCl, boiled to destroy the enzyme and coagulate the proteins, and filtered into a calibrated Pyrex test-tube. When exactly 5 c.c. of filtrate had been collected, 6 drops of phenol red

were added, and the *p*H was adjusted to 6·8. After the addition finally of 10 drops of urease, the amount of urea present was deduced, in the usual way, from the colour change in the next thirty minutes. The technique of these operations was precisely that already recommended for the determination of urea in elasmobranch blood.

"The results of this experiment, expressed as mgrms. of urea, are shown in Table I., and Fig. 2. The urea quantities there recorded arose, it should be emphasised, wholly from arginine: for in blank experiments the extract, even in undiluted form and in volumes as high as 1 c.c., yielded not the faintest indication of a pre-formed urea content appreciable by the method used."

TABLE I.

C.c. of diluted extract.	C.c. of original extract.	*p*H	Mg. urea in 5 c.c.
0·1	0·01	7·15	2·90
0·2	0·02	7·35	4·30
0·3	0·03	7·45	4·95
0·4	0·04	7·60	5·80
0·5	0·05	7·70	6·32
0·6	0·06	7·75	6·54
0·7	0·07	7·75	6·54
0·8	0·08	7·80	6·75
0·9	0·09	7·85	6·97
1·0	0·10	7·85	6·97

From the final *p*H value, by means of the graph, can be read off the amount of urea produced by the arginase under the standard conditions. From this, the unit of arginase can be determined on an arbitrary scale, a unit of arginase being defined as "that quantity of enzyme which, acting (under the conditions already defined) upon 6 c.c. of the standard substrate, will liberate in twenty-four hours one-tenth of the total urea potentially present—will produce, that is to say, a urea concentration of 1 mgrm. per 5 c.c."

Bonot and Cahn (*Bull. Soc. Biol. Chem.*, 1927, **9**, 1001; *Compt. rend.*, 1927, **184**, 246) have also made use of arginase for the determination of arginine. The urea produced is, however, estimated as crystalline dixanthylurea. A description of their procedure is appended:

"Three grms. of dry tissue, freed from extractives and grease, or 3 grms. of pure protein, are hydrolysed for forty-eight hours with 60 c.c.

of 20 per cent. HCl. The liquid is evaporated to dryness on a water bath *in vacuo* (20 mm. Hg) to remove the acid. The residue, taken up in warm water, is poured into a porcelain dish and boiled with 0·5 grm. of pure and active animal charcoal to remove the humic acid and decolorise the solution without loss of arginine. After filtration it is evaporated to dryness on a water bath to remove the last traces of hydrochloric acid. It is then taken up in water, bringing the volume to 250 c.c., and the reaction is adjusted to *p*H 9·9 (by means of 2·5*N* sodium hydroxide).

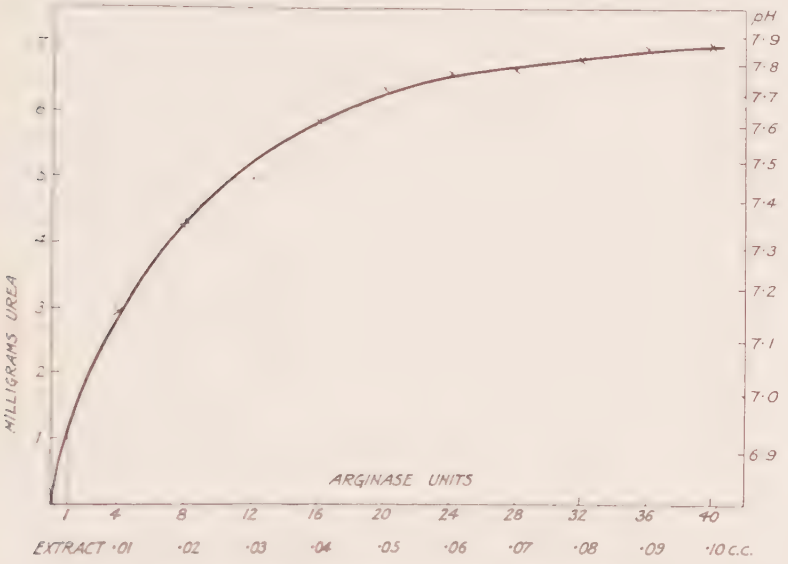


FIG. 2.—The Production of Urea from Arginase.

"To the clear liquid is added arginase. This is prepared by precipitation with acetone from the press-juice of dogs' liver, the activity being titrated against a solution of pure arginine (arginine carbonate prepared from scombrine from the ripe testes of mackerel). The activity of the dry powder is of influence in deciding the weight necessary to decompose completely a known quantity of arginase of the same order as that which is present in the sample to be determined. Excess of arginase must always be used. The fermentation is continued for seventy-two hours at 37°. The large amount of amino acids present act as buffers and, in consequence, the *p*H value does not change throughout the fermentation process. In a medium which does not contain mono-amino acids, an appropriate buffer must be used (1·5 grm. of glycine to the quantities

given above). The fermentation mixture is neutralised with dilute acetic acid, filtered, evaporated *in vacuo* on the water bath at a temperature below 70° to avoid hydrolysis of the urea. The residue is taken up in 70 per cent. acetic acid, a few drops of a protein precipitant are added to remove any traces of protein, and the mixture is centrifuged. Pour off into a beaker and precipitate with a 10 per cent. solution of xanthydrol in methyl alcohol. The condensation and precipitation of the dixanthylurea is complete in ten hours. Filter at the pump, wash the dixanthylurea with methyl alcohol saturated with xanthylurea, dry at 100° and weigh. Having obtained the figure for dixanthylurea, multiply by 0·414 to give the weight of arginine to which it corresponds."

This method has not yet been applied to many proteins, but an extended test of its value is much to be desired.

The enzyme method of determining units is in its infancy. Possibly the next step will be the determination of aspartic and glutaminic acids by means of asparaginase (for further information on asparaginase see Geddes and Hunter, *J. Biol. Chem.*, 1928, **77**, 197).

III. THE NATURE OF THE LINKAGES IN THE PROTEIN MOLECULE

The nature of the linkages which join up the different units of the protein molecule was for many years thought to have been settled by the work of Emil Fischer. Hofmeister, in 1902 (*Ergeb. d. Physiol.*, **1**, 759), had suggested that the peptide linkage in its keto form was probably the principal linkage present in proteins, and this probability was converted into a certainty by Fischer's work on the synthetic polypeptides (*Z. physiol. Chem.*, 1901 *et seq.*). It was shown that these substances, prepared in the laboratory by the condensation of the amino group of one amino acid with the carboxylic group of a second, were hydrolysed by the action of the pancreatic enzymes. Although Fischer explicitly stated that, in his opinion, other types of linkage might co-exist in intact proteins with the peptide linkage, little further work was done on the subject for nearly twenty years. In the last few years, however, the possibility of the existence of anhydride rings in the protein molecule has occupied the attention of many workers. In 1924, Abderhalden, pointing out the almost universal occur-

rence of diketopiperazines among the products of protein hydrolysis, put forward much evidence pointing to the pre-existence of these in the intact molecule (Abderhalden and Komm, *Z. physiol. Chem.*, 1924, **78**, 96). He has even suggested that proteins are not composed of molecules in the ordinary sense, but are aggregates of "elementary complexes" of anhydride rings (with or without amino acid side chains), held together by secondary valencies (*Naturwissenschaften*, 1924, **12**, 716).

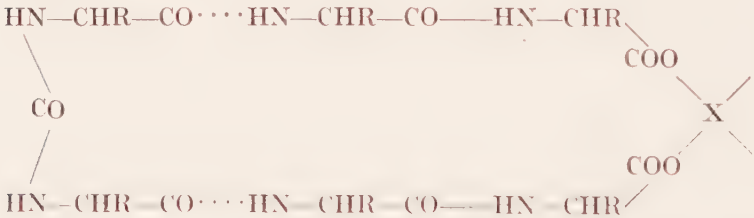
No reliable analytical methods are available for the determination of anhydride rings in the protein. Abderhalden and Komm (*Z. physiol. Chem.*, 1924, **139**, 181; 1924, **140**, 99) have used Jaffe's picric acid reaction for ketones as a qualitative test for the presence of diketopiperazine rings in intact proteins and have obtained positive reactions. Von Bitto's test with *m*-dinitrobenzene also gives positive reactions with some proteins (the ninhydrin reaction).

Blanchetière (*Bull. Soc. Chim.*, 1927, [iv], **41**, 101) has suggested that Siegfried's carbamate reaction can be used to determine diketopiperazines in the presence of amino acids and peptides. He states that 14 to 33 per cent. of nitrogen in commercial peptones formed by peptic and tryptic digestion, is present as diketopiperazines.

Zelinsky and Gawriloff (*Biochem. Z.*, 1927, **182**, 11) claim to be able to determine the anhydride nitrogen of proteins approximately by hydrolysing a 10 per cent. solution of the protein in 1 per cent. hydrochloric acid under pressure at 180°. The ratio of amino nitrogen (as determined by a formol titration) to the total nitrogen is claimed to be a measure of the anhydride nitrogen. Although it is well known that anhydrides are formed from dipeptides by heating in acid solutions, these workers produce evidence that anhydride formation does not occur under the conditions of their experiments. They obtain remarkably high figures for the anhydride nitrogen of proteins, as much as 40 per cent. of the total nitrogen being returned as anhydride for casein, and 30 to 50 (or possibly more) for gelatin.

The existence of other types of linkages, besides diketopiperazine or anhydride linkages, is not excluded by the known chemical

possibilities of the protein molecule. Bergmann (*Naturwissenschaften*, 1924, **12**, 1155) has pointed out that ester linkages may be formed at the hydroxyl groups of the hydroxyamino acids, and that the proximity of a hydroxylic grouping to an amino grouping may lead to interesting chemical transformations. He has made a number of interesting anhydrides, starting from dipeptides or diketopiperazines containing either serine or cystine. Certain of these anhydrides, known as the iso- or allo- forms, have colloidal properties, and in many respects resemble gelatin. On acid hydrolysis, they are converted into tetrapeptides. There is at present no evidence that any bodies resembling Bergmann's synthetic products do actually exist in the protein molecule. Nevertheless, the problem of the *rôle* of oxygen in the protein molecule is certainly an intriguing one. The proportion of oxygen to nitrogen obtained from the elementary analysis of a protein is nearly always greater than that obtained by a summation of that of the constituent amino acids in the proportions present. Brigl and Held (*Z. physiol. Chem.*, 1926, **152**, 280) have, therefore, suggested that ureide linkages may occur in proteins, joining long peptide chains.



The ureide linkage is supposed to occur between two amino groups at the ends of peptide chains, while an ester-like linkage, with an unknown constituent, has also to be postulated between the terminal carboxylic groups to preserve the neutral balance of the protein. This theory has received some support from Abderhalden and Kroner (*Z. physiol. Chem.*, 1927, **168**, 201), but it seems probable that, with increased knowledge of protein constitution derived from improved methods of analysis, no oxygen available for the formation of ureide bridges will be found.

A very lucid summary of the position reached in 1925 on the newer theories of protein structure has been given by Hunter (*Trans. Roy. Soc. Can.*, 1925, **19,** 1), and more recent and much fuller reviews by Vickery and Osborne (*Physiol. Reviews*, 1928, **8,** 393), and by Klarmann (*Die Rolle der zyklischen Aminosäureanhydride in der neueren Strukturchemie der Proteine*, 1929).

It is impossible to close the consideration of the possible linkages in the protein molecule without mentioning briefly some recent work on the denaturation of albumins and globulins. It has been shown that this change affects the linkages of the sulphur present in the protein. Raw egg-white, for example, gives no nitroprusside reaction, heat-coagulated egg-white gives a strong magenta pink (Harris, 1923, *Proc. Roy. Soc.*, B. **94,** 426); serum gives no nitroprusside reaction either raw or after heat-coagulation, but in the latter condition it will give a strong magenta pink if a strong reducing agent, such as sodium cyanide, is added with the sodium nitro-prusside and ammonia (Walker, *Biochem. J.*, 1925, **19,** 1082, 1085). Heat-coagulation has led, therefore, to the appearance, in the one case, of a sulphydryl; in the other, of a disulphide group. Denaturation of crystallised egg albumin is accompanied by a loss of water from the protein (M. Sörensen and S. P. L. Sörensen, 1923-25, *C. R. Lab. Carlsberg*, **15,** No. 9), and coagulation can be reversed by the action of dilute acids (Michaelis and Rona, *Biochem. Z.*, 1910, **29,** 294), dilute alkalis (M. Spiegel-Adolf, *Biochem. Z.*, 1926, **170,** 126), and certain lyotropic salts, such as potassium thio-cyanates (Wilhelm, *Koll. Z.*, 1929, **48,** 217). In the last case, the evidence is clear that reversal of coagulation is secured by the restoration of water to the protein. There is no evidence available as to whether agents which reverse coagulation also reverse the change in position of the sulphur groupings that accompanies denaturation. A consideration of the changes occurring in denaturation must be included in all discussions of protein structure, including any change in the relation of the protein to the proteolytic enzymes. Moreover, attractive as undoubtedly are the newer theories of protein structure, it must always be borne in mind that the relation of any postulated linkage to these enzymes must always remain the most

delicate test of its relation to naturally occurring proteins. Up to the present, diketopiperazines, esters and ureides have proved unsusceptible to hydrolysis by the proteolytic enzymes.

IV. THE ANALYSIS OF PROTEINS BY MEANS OF ENZYME ACTION

The proteolytic enzymes have been used as agents for the hydrolysis of proteins under laboratory conditions since the earliest days of protein chemistry, and the idea of the possibility of obtaining, by this means, evidence bearing on the distribution of the different units of protein structure throughout the large protein molecule is inherent in all the work recorded on this method. It has been made abundantly clear by the work of Dakin and Dale (*Biochem. J.*, 1919, **13**, 248) on the immune reactions of closely allied proteins, that not only the nature and the proportion of the different amino acids is of importance in determining the character of a protein, but also the order in which these are arranged in the molecule. No evidence on the order or structural pattern can be obtained from a complete hydrolysis, the products of which are the free amino acids. Even the small residue of diketopiperazines, almost invariably present at the end of a hydrolysis in a concentrated boiling solution of a mineral acid, can readily be hydrolysed further to give the free amino acids. Although the first stage in the development of an understanding of protein constitution had inevitably to be the examination of the products of a complete hydrolysis, a full knowledge and understanding of the architecture of the protein molecule can only be obtained by the examination of the stages of degradation.

In the early days of protein investigation, many attempts were made to isolate the products of the early stages of enzyme digestion. Much courage was expended on those efforts, but little was attained except to introduce into the literature a welter of new terms of vague meaning that are mostly best forgotten. In recent years, Wasteneys and Borsook (*J. Biol. Chem.*, 1924, **62**, 1) have defined a technique for separating the products of an incomplete

enzymic digestion of a protein into five fractions according to the complexity of their structure. These are :

Proteins. Soluble proteins are completely precipitated by the addition of trichloracetic acid to a concentration of 2 per cent. on the solution.

Metaproteins. The first stage apparent in an enzymic digestion of a protein is its partial conversion into a metaprotein. This change is a denaturation due to the acid of the peptic digest, and is not actually a stage of digestion (McFarlane, Dunbar, Borsook and Wastenеys, *J. Gen. Phys.*, 1927, **10**, 437). Adjustment of the digestion mixture to *p*H 6·0 leads to a complete precipitation of metaprotein.

Proteose. The first stages of enzymic degradation of a protein. Proteoses are not precipitated by trichloracetic acid, but are completely precipitated by saturation with anhydrous sodium sulphate at 33°. Proteoses can probably be regarded as complex polypeptides.

Peptones. These degradation products escape precipitation by any of the methods given above, but are completely precipitated in the presence of 14 per cent. of tannic acid, the reaction of the mixture being adjusted with standard (0·2*N*) sodium hydroxide to 7·0 and temperature kept below 20°. Peptones are probably simple polypeptides.

Sub-peptones.—This fraction left after removal of the more complex fraction consists of peptides and simple amino acids. Probably only some of the lower peptides, di-, tri- and tetrapeptides escape precipitation by the methods given above. These can be precipitated by alcohol after adjustment of the *p*H value of the solution (Folin and Denis, *J. Biol. Chem.*, 1912, **11**, 529; Van Slyke and Meyer, *J. Biol. Chem.*, 1912, **12**, 399).

There is very little doubt that the examination of the proteose and peptone fractions of protein digests with the methods and knowledge now available would shed great light on the way in which the amino acids are grouped in the protein molecule. Some progress has already been made in this direction, and a number of

interesting peptides isolated from enzymic digests of proteins are enumerated below.

[For an account of the polypeptides and peptic anhydrides isolated from the products of acid hydrolysis, see Klarmann (*loc. cit.*) and Vickery and Osborne (*Physiological Reviews*, 1928, **8**, 393).]

Glycylproline anhydride has been isolated by Abderhalden and Komm (*Z. physiol. Chem.*, 1925, **145**, 308) from a pancreatic digest of edestin.

Phospho-peptone of casein (nonapeptide phosphoric acid ester), consisting of 4 molecules of α-amino-β-hydroxybutyric acid, 3 molecules of hydroxyglutaminic acid, 2 molecules of serine, and 3 molecules of phosphoric acid, has been isolated by Rimington (*Biochem. J.*, 1927, **21**, 1179, 1187) from a tryptic digest of casein.

Tyrosine-2-glutamine-glutaminic acid has been isolated from a peptic digest of gliadin by Nakashima (*J. Biochem. Japan*, 1926, **6**, 55).

Tyrosine-proline has been isolated from a tryptic digest of casein by Abderhalden and H. Sickel (*Z. physiol. Chem.*, 1925, **144**, 80; 1926, **153**, 16).

The use of proteolytic enzymes as analytical tools is likely to increase considerably in the future. The methods of obtaining preparations of individual enzymes free from other enzymes have only within recent years been brought into a satisfactory condition, largely by the work of Willstatter and of Waldschmidt-Leitz and his colleagues. The methods of preparing enzymes worked out by them will be of the greatest value, if adopted generally, in preventing the confusion of evidence that may arise if impure preparations are used.

The proteolytic enzymes of the tissues of animals and plants have been grouped for convenience into three classes, corresponding to the three well-known digestive enzymes from the stomach (pepsin), pancreas (trypsin) and intestinal mucosa (erepsin) of the vertebrate alimentary track.

Pepsin. This enzyme is a proteinase and acts only in acid solutions (pH at optimum activity, 2). It has the power of attacking all native proteins with the exception of the keratins.

No free amino acids ever appear in a peptic digest. During peptic digestion amino and carboxylic groups appear in exactly equivalent proportions (Sørensen and Katschioni-Walther, *Z. physiol. Chem.*, 1928, **174**, 251). This fact is not inconsistent with the suggestion that pepsin may open up anhydride rings in the protein. It puts out of court the suggestion that pepsin may open up ether or ester linkages (Sadikoff, *Biochem. Z.*, 1926, **179**, 326). Pepsin breaks the protein into a number of primary degradation products which undergo little further breakdown by this enzyme. Pepsin breaks the molecule mainly into large aggregates, but bodies as simple as tetrapeptides have been isolated from peptic digests. By the action of pepsin, many more linkages are made available to the action of pancreatic trypsin. In many proteins about 20 per cent. of the total potential amino nitrogen is converted into free amino nitrogen in peptic digestion. Pepsin is generally prepared from the gastric mucosa. Willstätter and Bamann consider that this tissue yields two enzymes, both proteinases, the first *pepsin*, with an optimal activity at *p*H 2, the second *lathepsin*, with optimal activity at *p*H 3·5 to 4·0 for most proteins, *p*H 5·0 for clupein (*Z. physiol. Chem.*, 1929, **180**, 127).

Trypsin. The mixture of pancreatic enzymes usually referred to as trypsin acts in weakly alkaline solutions, the *p*H at optimal activity being 8·0. It has some power of hydrolysing native proteins, releasing about half the total amino nitrogen, but if allowed to act after pepsin, it generally carries the production of free amino acid groups to 70 per cent. of the total potential amino nitrogen. There is a copious production of free amino acids during a digestion with pancreatic trypsin. It is generally accepted that this mixture of enzymes attacks the peptide linkage within certain limiting conditions not yet exactly defined, but is incapable of hydrolysing dipeptides. According to Abderhalden and Koppel (*Fermentforsch.*, 1928, **9**, 516), the tripeptide from *l*-cystine is also immune from tryptic action, the nona-peptide completely hydrolysed and the intermediate peptides attacked in varying degrees. The tetra-peptide *d*-alanyl-*l* leucyl glycyl alanine is slowly attacked by trypsin, and the pentapeptide glycyl-*d*-alanyl-*l*-leucyl-glycyl-alanine is rapidly attacked (Abder-

halden, Brockmann and Sickel, *Fermentforsch.* 1928, **9**, 446, 462). The action of the trypsin apparently increases with the complexity of the peptide.

Waldschmidt-Leitz and his collaborators (*Z. physiol. Chem.*, 1925, **149**, 203) have distinguished between three pancreatic enzymes, *trypsin*, *trypsin-kinase* and pancreatic *erepsin*. According to them, trypsin-kinase can hydrolyse all proteins, including protamines, histones and peptone, but not dipeptides; trypsin can hydrolyse protamines, histones and peptone, but not other proteins nor dipeptides; erepsin only attacks dipeptides.

The method employed by Waldschmidt-Leitz and his co-workers of separating the three pancreatic enzymes consists in a series of fractional adsorptions followed by the elution of the enzyme with special solutions. In an acid solution (*p*H 4·7 to 3·8) erepsin is adsorbed by precipitated aluminium hydroxide, but trypsin and trypsin-kinase are left in solution. Erepsin is recovered from the absorbing alumina by extraction with alkaline phosphates at *p*H 8·2 or with 0·04*N* ammonia in the presence of 20 per cent. glycerin (Waldschmidt-Leitz and Harteneck, *Z. physiol. Chem.*, 1925, **147**, 286). Trypsin and trypsin-kinase are adsorbed by alumina in a neutral solution and can be separated by selective elution, the latter being extracted with a 1 per cent. solution of casein, the former with 0·04*N* ammonia in 20 per cent. dilution of glycerin (Waldschmidt-Leitz, Schaffner and Grassmann, *Z. physiol. Chem.*, 1926, **156**, 68). The alumina used for adsorption is precipitated as a hydroxide, $Al(OH)_3$, a form which is unstable, but is allowed to stand before use until it has passed into the stable form referred to as "C_2" (Waldschmidt-Leitz, Schaffner and Grassmann, *Z. physiol. Chem.*, 1926, **156**, 68; and Willstatter, Kraut and Erbacher, *Ber.*, 1925, **58**, 2448; also Waldschmidt-Leitz and Harteneck, *Z. physiol. Chem.*, 1925, **149**, 203).

The method of separating the three pancreatic enzymes is as follows:

A pig's pancreas is dried with acetone and ether and the dried gland is extracted with 87 per cent. glycerin. Twenty c.c. of the glycerin extract is diluted with an equal volume of water and brought to a *p*H value of 3·8 by means of an acetate buffer. Forty c.c. of the dilution

are shaken up four times with a suspension of alumina (referred to under a laboratory index "C_2"), each c.c. of the suspension containing 35 mgrms. Al_2O_3. The *erepsin* is obtained from the adsorbate by elution with 0·04*N* ammonia in 20 per cent. glycerin. The filtrate from this preparation contains trypsin free from erepsin. The trypsin is freed from activated trypsin (trypsin-kinase) by adjusting the *p*H of the filtrate to 7·0 with normal NH_3 solution. Thirty c.c. are twice shaken up with 7·0 c.c. alumina "C_2" (245 mgrms. Al_2O_3). Elution of the second absorbate with 30 c.c. of a neutral 1 per cent. solution of casein in 20 per cent. glycerin gives *trypsin-kinase*. After twice washing the extracted adsorbate with 20 c.c. of 20 per cent. glycerin, a second elution with 30 c.c. of 0·04*N* NH_3 in 20 per cent. glycerin gives *trypsin* free from kinase (Waldschmidt-Leitz, Schäffner and Grassmann, *Z. physiol. Chem.*, 1926, **156**, 68).

Waldschmidt-Leitz and his colleagues are engaged in an important series of investigations on the nature of the attack of these pure enzyme preparations on proteins that is likely shortly to lead to important new knowledge on the nature of the linkages in the protein molecule (Waldschmidt-Leitz, Schäffner and Grassmann, *Z. physiol. Chem.*, 1926, **156**, 68).

For a discussion on the proteolytic enzymes of autolysed yeast, see Grassmann and Dyckerhoff, *Z. physiol. Chem.*, 1928, **179**, 41; 1928, **175**, 18; Willstätter and Grassmann, *ibid.*, 1926, **153**, 250. For bacterial enzymes, see Stephenson (1930).

Erepsin. This enzyme is usually prepared from the intestinal mucosa. It acts in weakly alkaline solutions, the *p*H of optimal activity being very close to that of trypsin. Its action is limited to the hydrolysis of the simplest peptides. It hydrolyses all known dipeptides, some tripeptides and even tetrapeptides. It is agreed, on all sides, that erepsin hydrolyses the peptide link. The reaction follows the course of a monomolecular reaction (Levene, Bass and Steiger, *J. Biol. Chem.*, 1929, **81**, 221). Waldschmidt-Leitz, Grassmann and Schäffner (*Ber.*, 1927, **40B**, 359) record that pig erepsin hydrolyses luciamide. Linderstrøm-Lang (*Z. physiol. Chem.*, 1929, **182**, 151) considers that intestinal erepsin contains two dipeptidases with *p*H of optimal activity at 7·3 and 8·1, respectively.

Asparaginase (Amidase). This enzyme acts in weakly alkaline solutions, the *p*H of optimal activity being 8·0, and, therefore,

very close to that of trypsin and erepsin. Geddes and Hunter (*J. Biol. Chem.*, 1928, **77**, 197) have prepared an active concentrated preparation of this enzyme from ground yeast cells by extraction with 50 per cent. glycerin and precipitation with safranin. This enzyme is said only to act as an amide-splitting agent for the two amino acid amides, asparagine and glutamine. Some proteolytic activity is found in the preparation. It seems possible that the amide link is to be regarded as a special case of the peptide link (Grover and Chibnall, *Biochem. J.*, 1927, **21**, 857) and that amidases are, therefore, indistinguishable from erepsin.

Phosphoric Acid Esterase. The phosphoric acid esterase, found by Robison in bone extract, hydrolyses the monopeptide of casein with release of phosphoric acid (Rimington, *C. R. Lab. Carlsberg*, 1927, **17**, No. 2).

The progress of an enzymic hydrolysis of a protein is frequently followed by the estimation of the free carboxylic and the free amino groups. The determination of both these groups is now possible by volumetric methods. Foreman (*Biochem. J.*, 1920, **14**, 451) has shown that carboxylic groups can be accurately titrated by alcoholic potash in the presence of organic bases if the titration be carried out in solutions containing 81 per cent. (or more) of alcohol, phenolphthalein being used as indicator. A method based on similar principles has been described by Willstatter and Waldschmidt-Leitz (*Ber.*, 1921, **54**, 2988). Linderstrom-Lang (*C. R. Lab. Carlsberg*, 1927, **17**, No. 4) has described an adaptation of the same principle to the titration of free amino groups in the presence of organic acids, the titration being carried out with alcoholic hydrochloric acid in the presence of 100 to 200 c.c. of acetone (99 per cent.) to 10 c.c. of water, naphthyl red (benzene-azo-α-naphthylamine turning between pH 3·7 and 5·0) being used as indicator. For full working details of these methods, the reader is referred to the original papers. The methods have been applied to a number of investigations on the progress of the hydrolysis of a protein by enzyme action (see especially Sorensen and Katschioni-Walther, *C. R. Lab. Carlsberg*, 1928, **17**, No. 7; Foreman, *Biochem. J.*, 1928, **22**, 208).

V. THE SIZE OF THE UNIT AGGREGATE

An analysis of a protein will give considerable information as to the amino acids from which it is constituted, and as to the percentage of each amino acid present. Assuming that at least 1 molecule of the constituent present in lowest amount must be combined into the protein, it is possible to calculate for the latter a minimum molecular weight. In this way Jordan Lloyd, in 1920 (*Biochem. J.*, **14**, 147), calculated the minimum molecular weight of gelatin to be 10,300: Cohn, Hendry and Prentiss (*J. Biol. Chem.*, 1925, **63**, 721) have more recently gathered together all the evidence available up to 1925 on the determination of special groupings or special atoms (such as the iron in hæmoglobin) and have used the figures, together with the combining weights for acids and alkalis (obtained by titration) and certain physico-chemical measurements, such as osmotic pressure determinations, for determining the molecular weights of proteins. Their paper gives a full review of the literature and the values recorded by them are tabulated below:

Protein.	Minimal combining weight.
Gelatin	10,300
Zein	19,400
Gliadin	20,700
Hæmocyanin (limulus)	22,700
Bence-Jones's protein	24,500
Edestin	29,000
Hæmocyanin (octopus)	33,500
Egg albumin	33,800
Glutenin	36,300
Fibrin	42,000
Serum albumin	45,000
Hæmoglobin (dog)	50,000
Serum globulin	81,000
Casein	192,000

The values obtained for the minimal combining weights are in some cases a little startling, particularly those given for serum globulin and casein. Recently, however, Sørensen, Linderstrøm-

Lang and their colleagues have brought forward considerable evidence to show that neither serum globulin nor casein are to be regarded as individual proteins, but rather as complexes formed by the association of two or more proteins with similar properties (Sorensen, *C. R. Lab. Carlsberg*, 1923 25, **15**, No. 11 ; Linderstrom-Lang and Kodama, *C. R. Lab. Carlsberg*, 1925 27, **16**, No. 1 : Linderstrom-Lang, *Id.*, 1929, **17**, No. 9). Whether other proteins now regarded as individuals will in future have to be regarded as associated protein complexes is not yet known.

The hypothesis that serum globulin and casein are protein complexes is not to be confused with the view that has been put forward by other workers, that proteins are not to be regarded as having molecules built up of atoms held together by primary valencies, but as being rather more in the nature of constant composition mixtures, in which primary molecules (diketopiperazines) are held into aggregates by secondary or residual valencies. Whatever view may be held, however, it is obviously of great interest to know what may be the weight of the smallest particle (molecule, aggregate or complex) which can exist as a kinetic unit and which will also possess the properties characteristic of the proteins as a class. The size of the primary particle is of great significance when it comes to a consideration of the fundamental basis of diffusion and the properties which proteins display in the presence of semi-permeable membranes. The calculation of the molecular (or primary particle) weights of proteins, either from the results of analysis or by the application of physical measurements, obviously demands a high state of purity in the material to be used. Purification of protein preparations by fractional precipitation, differential solubilities, differential adsorption, dialysis and, in a few cases, by crystallisation has been in common use for a considerable time. Within recent years, electrodialysis as a method for the separation and purification of the proteins has come largely to the fore.

Electrodialysis as a Method of Protein Purification

The combined use of dialysis and a fall of electric potential as a method of protein purification was first used by Dhéré as long

ago as 1910 (Dhéré, *Compt. rend.*, 1910, **150**, 993; Dhéré and Gorgolewski, 1910, **150**, 934; see also Morse and Pierce, *Z. physikal. Chem.*, 1903, **45**, 606). Dhéré has recently given an account of his method in the light of seventeen years' experience of its use (*Koll. Z.*, 1927, **41**, 245). Essentially the method

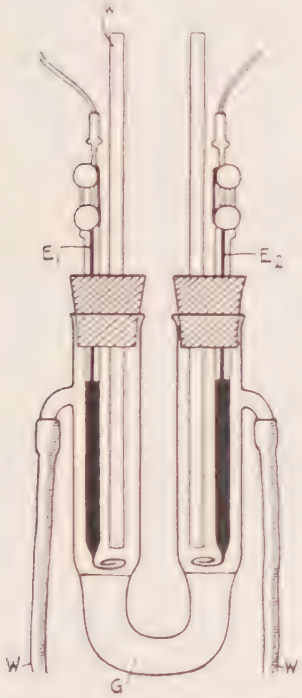


FIG. 3A.—E_1, E_2. Electrodes; G. Gelatin Gel: W. Water circulation.

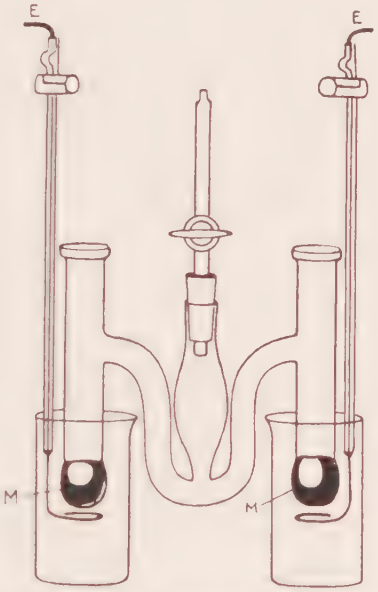


FIG. 3B.—EE. Electrodes; MM. Membranes.

consists in placing a protein solution in a closed vessel with two semi-permeable walls, each of which abuts on to a vessel containing an electrode (positive and negative respectively), and through which pure water can circulate freely. The solution in the closed middle vessel, therefore, undergoes dialysis in an electric field when the electrical circuit is closed and the water is flowing through

the two outer vessels. It is important to notice that this circulation of the water not only removes dialysable impurities from the protein, but also removes the acid and alkali produced during electrolysis at the positive and negative poles, respectively. More-

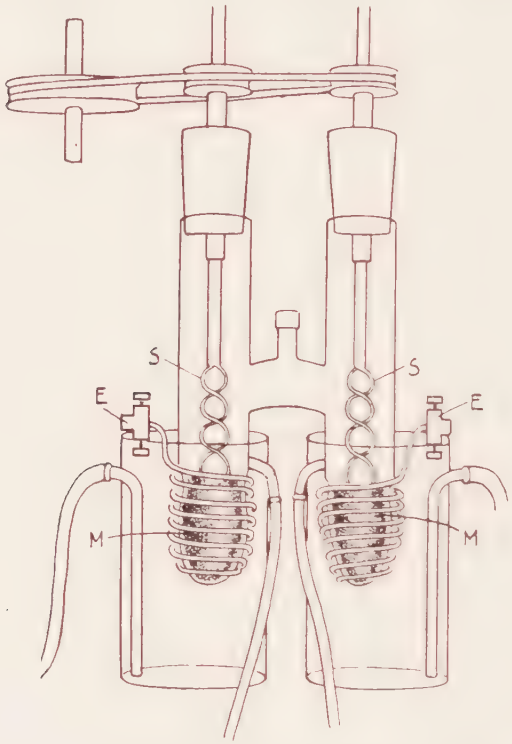


Fig. 3c.—E. Electrodes; M. Membranes; S. Stirrers.

over, it keeps the system cool. In Figs. 3A, 3B and 3C are shown three types of apparatus used by Dhéré.

Fig. 3A represents an early type used for the electrodialysis of a gelatin gel. The gel is in the narrow section of the U-tube, and gel surface forms the permeable membrane. Fig. 3B represents a type from which the non-dialysable protein can be obtained in three fractions — an anode fraction, a cathode fraction

and a middle fraction. Fig. 3c represents a very efficient type of electrodialyser, in which the solutions in the middle chamber can be kept constantly stirred, a device which reduces the trouble sometimes encountered due to the polarisation of the membrane and which greatly accelerates the purification. In the two latter types of apparatus, collodion membranes in the form of sacks separate the two branches of the inner vessel from the two outer vessels.

Innumerable patterns of electrodialysers have been described in the literature. The apparatus used by Pauli (*Biochem. Z.*, 1924, **152**, 357) is shown in Fig. 4. A convenient, though elabo-

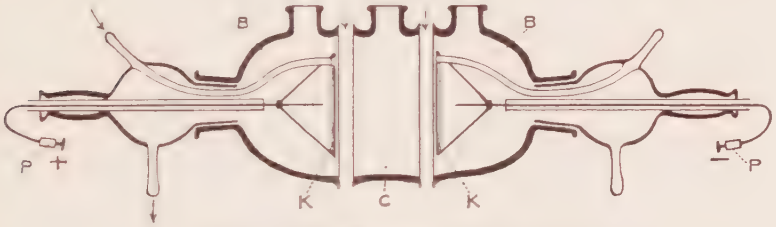


FIG. 4.—Electrodialyser used by Pauli.

rate, model with a large area of membrane is described by Fricke, Fischer and Borschers (*Koll. Z.*, 1926, **39**, 152, 371); a convenient and very simple pattern by Reiner (*Koll. Z.*, 1926, **40**, 123). This is illustrated in Fig. 5. Knaggs and Schryver have described an apparatus (shown in Fig. 6) suitable for handling large volumes of solutions (*Biochem. J.*, 1924, **18**, 1079), and Baer (*Koll. Z.*, 1928, **46**, 176) an apparatus adapted for use with quantities as low as 2 c.c. (Fig. 7). The three points of technical interest in the setting up of any type of apparatus are, the choice and preparation of the membranes, the regulation of the potential and amperage of the electric current, and the regulation of the water circulation.

Membrane.—Semi-permeable membranes for dialysis must be sufficiently tough to stand reasonable handling, and must, of course, be free from leakage. Collodion membranes are frequently used, since they can readily be prepared in any shape desired.

They can be cast on glass apparatus, and if very large membranes are desired, they can be strengthened by the incorporation of

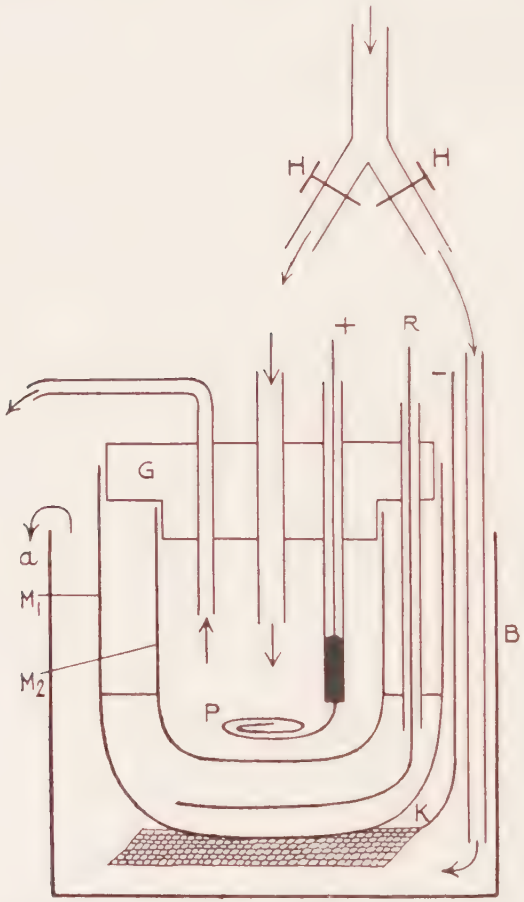


Fig. 5.—Electrodialyser used by Reiner. H. Stopcocks for water flow. K and P. Electrodes; M_1 and M_2. Membranes; R. Stirrer.

muslin. Parchment membranes have also been used successfully (Pauli, *Biochem. Z.*, 1924, **152**, 355). Parchment membranes tend to acquire a negative charge, and collodion membranes also become

polarised under some conditions. Ettisch, Bradfield and Ewig (*Koll. Z.*, 1928, **45**, 141) recommend the use of a parchment at the kathode with a collodion membrane coated with serum albumin

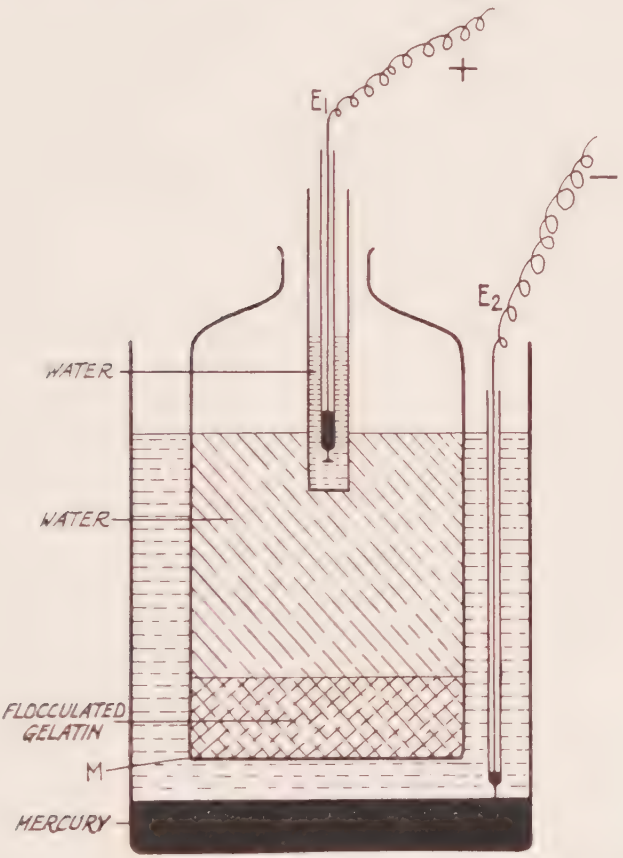


FIG. 6.—Electrodialyser used by Kraggs and Schryver. E_1, E_2. Electrodes; M. Membrane.

at the anode in the dialysis of serum, and state that this system is non-polarisable. The whole question of the polarisation of membranes in electrodialysis is discussed by Reiner (*loc. cit.*).

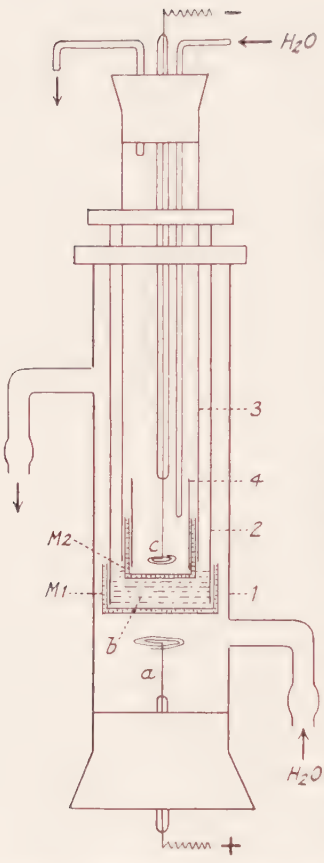


Fig. 7. Electrodialyser used by Baer. *a* and *c*. Electrodes; *b*. protein solution; M_1 and M_2. Membranes; 1 4. Glass containers.

Reiner's method of preparing the large collodion sacks for use with the apparatus shown in Fig. 5 is as follows :—

"Some 4 per cent. solution of collodion in ether-alcohol is poured into a beaker of 400 or 600 c.c. capacity, and the beaker is rotated by hand for five minutes; after draining off the excess, the beaker is again turned for five minutes and then dried in air for half an hour to twelve hours, and finally treated with 90 per cent. alcohol for from one to thirty minutes. It is then washed out several times with distilled water, and the membrane is carefully separated from the edge of the beaker. The membrane is now ready for use, but in most cases was sterilised. For this purpose it was filled with water and mounted on the rubber stopper (see Fig. 5), immersed in distilled water and boiled for half an hour."

Electric Current.—At the start of an electrodialysis, the amount of current passed should be low. Pauli gives a preliminary purification by ordinary dialysis and then electrodialyses with a current of not more than 0·3 to 0·8 milliampère per sq. c.c. of membrane. Purification is completed at a voltage of 440. Forty-eight hours should be sufficient time to complete the purification. Reiner recommends starting at a voltage of 50 and regulating the flow of current so that at first 400 to 500 milliampères flow through the system. Towards the end, the voltage should be raised to 110 with a stream of 3 to 4 milliampères. Dhéré uses 130 volts potential throughout, but in that case the flow of current must be regulated with an external

resistance. An excess of current passing will cause a rise of temperature, and the advantages gained by removing electrolytes will be lost by the production of protein degradation products. A sufficient circulation of water round the electrodes will help to keep the system cool, and, if necessary, ice must be added. Platinum must be used for the anode, but the cathode is frequently made from copper.

As a method of purifying proteins, electrodialysis has the advantage of speed of action with a consequent lessening of the risk due to putrefactive organisms. It gives preparations free from all ionogenic impurities. It appears, however, that it may lead to a separation of a protein into fractions. Schryver and Thimann (*Biochem. J.*, 1927, **21**, 1284), for instance, find that by this means gelatin is separated into two constituents which cannot be recombined to form the original protein, and Macheboeuf, Sorensen and Sørensen (*C. R. Lab. Carlsberg*, 1925 27, **16**, No. 12) that crystalline egg albumin is separated into two fractions differing in their phosphorus content.

The advance made by the method in recent years is evidence of its utility. Gelatin as ordinarily prepared by electrodialysis does not, however, differ in any particular from gelatin prepared by extraction with acid solutions of sodium chloride and subsequent dialysis (Dhéré, *Koll. Z.*, 1927, **41**, 245; Pleass, unpublished).

Physical Methods in the Determination of the Size of the Primary Particle of Protein

A number of physical methods have been used for determining the mass of the unit aggregate or primary particle of protein. Obviously, for measurements so obtained to have any value, both the protein and the solvent used must be in a very high state of purity.

Adair (*Proc. Roy. Soc.*, A, 1925, **108**, 627) has used osmotic pressure measurements to determine molecular weight of several proteins. He has worked out very fully the experimental condi-

tions which have to be observed in order to obtain steady and consistent readings, and finds that working at a temperature of 0°, osmotic pressures which are constant for long periods are obtained with solutions of proteins. He finds that under many circumstances the results are very little influenced by membrane equilibria and that accurate values are obtained for the molecular weights of proteins by using Dalton's Law of partial pressures as a basis of calculation. The theoretical considerations are discussed fully in a recent paper (Adair, *Proc. Roy. Soc.*, A, 1928, **120**, 573). Adair gives the following figures for the molecular weight of a number of proteins (*loc. cit.* and *Skandinav. Archiv.*, 1926, **49**, 76, and *J. Amer. Chem. Soc.*, 1927, **49**, 2524).

Hæmoglobin . . .	68,000
Serum albumin . .	62,000
Pseudoglobulin . .	130,000 to 150,000
Euglobulin . . .	174,000
Ovalbumin . . .	43,000

Svedberg and his colleagues have used the ultra-centrifuge for determining the mass of unit aggregates. Svedberg gives the value of 62,000 to 71,000 for hæmoglobin and 34,000 for egg albumin (Svedberg and Fåhraeus, *J. Amer. Chem. Soc.*, 1926, **48**, 430; Svedberg and Nichols, 1926, *J. Amer. Chem. Soc.*, **48**, 3081). These figures are very close to the values obtained by Adair. It has been demonstrated, therefore, that the particle of protein which acts as a unit in the terms of the kinetic theory has a considerable mass.

Another physical method which has been used to determine molecular weights is the lowering of the freezing point of liquid phenol as a result of dissolving proteins in the system. The first recorded results appeared to indicate that, under these conditions, the protein became dispersed in solution in the form of molecules with a molecular weight of between 200 and 400 (Troensegaard and Schmidt, *Z. physiol. Chem.*, 1924, **133**, 116; Herzog and Kobel, *Z. physiol. Chem.*, 1924, **134**, 296). Cohn and Conant, however, have repeated similar experiments and come to the conclusion that the low values obtained were due to the pre-

sence of moisture in the system (*Proc. Nat. Acad. Sci.*, 1926, **12**, 433).

The evidence of the mass of the protein molecule available from physical measurements is, therefore, that the primary particle is very large and that the molecular weight is of the order of 10^5 to 10^6.

The method of X-ray analysis has been applied to proteins, but the work is not very far advanced. Scherrer (*Machr. Ges. Wiss. Gottingen*, 1918, 96) examined gelatin gels by this method and found no evidence of crystal structure. Katz and Gerngross (*Koll. Z.*, 1926, **39**, 181) have found evidence of crystal structure in strongly stretched gelatin gels and in collagen fibres from tendons. No estimate of the mass of the crystals was possible on account of the vagueness of the pattern. Brill (*Ann.*, 1923, **434**, 204) has examined silk fibroin, and considers that the X-ray diagram indicates the repetition in the crystal of a unit having a weight of between 500 and 660. At present this observation stands alone.

VI. CONCLUSION

Recent advances in the analysis of the proteins have resulted in the discovery of several new amino acids. The outstanding characteristic of these, as a group, is that most of them are hydroxy acids corresponding to simple amino acids already previously known. In addition, sulphur has been shown to exist in a previously unrecognised form of combination, and the appearance of a positive test for sulphide groups in cystine-containing proteins has been shown, in the case of the albumins and globulins, to be related to the state of the protein as undenatured and denatured. Recent research has confirmed the overwhelming importance of the peptide link in protein structure. Anhydride linkages undoubtedly exist, which lead to a more compact molecule, especially in the case of such proteins as gelatin, collagen or the keratins, and an ester linkage has been found in casein.

Possibly the most significant fact revealed by recent work is the establishment of the disaccharide, glucosamine-mannose, as

a unit in the molecule of the albumins and the globulins of horse-serum and of egg-white and egg-yolk. The nature of the linkage which joins this unit to the protein molecule and its function in the molecule are at present unknown. The possibility that a chemically active substance, like a sugar, may be a normal constituent of cell proteins is biologically of great interest. It seems possible that in their naturally occurring state in the living organism, the proteins exist in association with some chemically active complex, and that the sensitiveness of the proteins to physical change provides a mechanism whereby a control can be effected through the environment on the chemical activity of these associated groups. If animal proteins are considered, it will be seen that protamines and histones occur in nature only as salts of nucleic acid; albumins and globulins possibly only in association with a carbohydrate group; mucoproteins as esters of chondroitin sulphuric acid; chromoproteins as a complex of a protein with a respiratory pigment. Even casein has been shown to be associated with phosphoric acid through an ester linkage, and a carbohydrate has been isolated from wool keratins. Among animal proteins, therefore, only the connective tissue proteins (collagen, etc.) have escaped the implication that some group other than an amino acid is present in the unit complex as it occurs in nature. These proteins, however, which consist largely of glycine, probably have a purely physical (skeletal) *rôle* in the mechanism of the body, and play no part in the cycle of chemical change that maintains the life of the organism. Of the proteins of plant cells, too little is known at present for any generalisation to be attempted.

In conclusion, it is important to notice that in considering the value of proteins as food materials for the living (and particularly the growing) animal, the knowledge obtained by a chemical analysis does not carry the matter very far forward. While certain units must be present in a "good" food protein, the availability of any unit for absorption and synthesis is not indicated as yet by any known method of analysis. For the assay of the value of a protein from the biological standpoint, recourse must still be had to biological methods.

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BIOLOGICAL ANALYSIS OF PROTEINS

By Harriette Chick, D.Sc.

While plants can synthesise proteins from the inorganic constituents of their food, animal cells require their nitrogen to be supplied as an assorted mixture of amino acids. These are necessary to repair the daily nitrogenous loss, and, in case of the young, to provide material for building the growing tissues. The amount of amino acids derived from the proteins contained in an ordinary diet is much in excess of these requirements, and the surplus is de-aminised and utilised as fuel.

The protein ingested must contain most of the amino-acids of which the animal's tissues are composed, and since proteins of meat, milk and eggs resemble those of other animal tissues more nearly, as regards the nature and relative amount of the constituent amino-acids, than do those derived from plant tissues (vegetables, cereals, legumes), it is generally true that nitrogenous equilibrium can be attained with a smaller daily intake of animal than of vegetable proteins; in other words, the former have a higher "biological value." In theory the flesh of the animal's own species would possess the maximum biological value, and this has been shown to be true by Michaud (1909) in experiments with the dog.

Our knowledge of the relative "biological value" of various proteins for the nutrition of mammals is based on experimental work of two types, each of which possesses considerable technical difficulties: (1) comparison of the minimal amounts of various purified proteins necessary in a diet in order that young animals may be reared to maturity; (2) determination of the minimal amounts necessary to maintain nitrogenous equilibrium in a metabolic experiment lasting a few days or weeks. In either case the protein in question forms the sole source of nitrogen in the dietary.

Relative Value of Proteins for Rearing Young Animals to Maturity

Seeing that these experiments extend over long periods of time and that the criterion is the normal growth and reproduction of the experimental animal, it is essential that the diet shall contain all essential elements other than the protein under investigation. If any other food factor, such as vitamins, minerals, etc., be defective, the failure to thrive may be erroneously attributed to protein deficiency. In some of the earlier experiments on the nutritional value of proteins it is certain that the vitamin supply was defective, and, indeed, the impossibility of rearing young animals on purified foodstuffs led to the rediscovery of accessory food factors by Stepp, Hopkins, Osborne and Mendel; in others, it is likely that the nutritive value of the proteins studied may have been overestimated owing to contamination with vitamins.

The most complete investigations in this field are those of Osborne and Mendel and their colleagues, who, working with young growing rats, tested a large series of purified proteins and determined the minimal amounts necessary to maintain normal growth. Some of their results are summarised in Table I.

In many instances the failure of certain proteins to support growth has been traced to shortage or absence of a particular amino acid and has been repaired when the latter has been added to the diet. Zein, a protein of maize, contains neither tryptophane nor lysine, and can neither maintain weight nor support growth. Willcock and Hopkins (*J. Physiol.*, 1906, **35**, 88) and Osborne and Mendel (*J. Biol. Chem.*, 1914, **17**, 325), working with young mice and rats respectively, found that the body weight fell progressively on diets containing as much as 18 per cent. zein but could be maintained if a small proportion (0·5 per cent.) of tryptophane were added to the diet, while the further addition of lysine (0·5 per cent.) was needed before growth could take place. The indispensability of lysine for growth has also been shown in experiments with wheat gliadin, a protein which contains only an insignificant amount of this amino acid. Rats which failed to grow on diets containing 18 per cent. of gliadin grew

normally when 0·5 per cent. of lysine was added (Osborne and Mendel, 1914). Casein is a protein containing but small amount of cystine: 12 per cent. in the diet was found necessary to maintain normal growth, but 9 per cent. sufficed if 0·5 per cent. cystine was added. The absence of glycine (glycocoll) in a protein (*e.g.*, casein, lactalbumin) is immaterial, for the animal body is able to synthesise this amino acid for itself.

Table I

"*Biological value*" *of various proteins, determined by minimum proportions necessary in diet to maintain growth of young rats* (Osborne and Mendel and others, *J. Biol. Chem.*, 1914, **17**, 325; **18**, 1; 1915, **20**, 351; **22**, 242; 1916, **26**, 1).

Protein.	Amino acids absent, or present in very small amount.	Amount required in diet to maintain growth.			
		If sole source of nitrogen.		If supplemented with	
		Protein per cent.	Character of growth.		Protein per cent.	Character of growth.
Lactalbumin	Glycine	8	Normal			
Caseinogen	Glycine, cystine	12	,,	Cystine, 0·5%	9	Normal
Edestin (hemp seed)	Lysine, cystine	15	,,	Lysine, 0·4–0·8%	11–12	,,
Gliadin (wheat)	Glycine, lysine	18	Very poor	Lysine, 0·5%	17·5	,,
Zein (maize)	Glycine, tryptophane, lysine	18	No growth, fall in weight	Tryptophane, 0·5% / Lysine 0·5%	17	..
Glutelin (maize)	—	18	Normal	—		
Glutenin (wheat)	—	9	Subnormal	—		
Ovo vitellin / Ovalbumin (hen's egg)	—	9	,,	—		
	—	9	..	—		

The biological value of the protein mixtures derived from single natural foodstuffs has also been determined by the use of a similar method. Diets containing 20 per cent. of protein derived from white wheaten flour are unable to maintain normal growth in young rats, whereas on diets containing 10 per cent. of whole-wheat proteins, normal growth and fertility are secured, although the young are not satisfactorily reared (Osborne and Mendel, *J. Biol. Chem.*, 1919, **37**, 557; McCollum and co-workers, *J. Biol. Chem.*, 1921, **47**, 235). Normal growth and reproduction has, however, been observed with rats fed on the proteins (14 per cent. in diet) of ox kidney, ox liver or ox muscle (McCollum and co-workers, *J. Biol. Chem.*, 1921, **47**, 111).

Working with diets in which the protein supply was limited to 9 per cent., McCollum and his colleagues (*loc. cit.*, 1921, 2) arranged the foodstuffs examined in the following order, as regards the biological value of their constituent proteins :—

(1) Beef kidney.
(2) Whole wheat.
(3) Milk ; beef liver.
(4) Beef muscle ; barley ; rye.
(5) Maize ; oats.
(6) Soya bean ; navy bean ; pea.

Relative Value of Proteins for Maintaining Nitrogenous Equilibrium

Experiments of this type have been carried out on dogs, pigs, rats and man, and, with a few exceptions, the proteins investigated have been the mixtures occurring in natural foods. The biological value is measured by the minimum daily intake of a particular protein necessary to maintain nitrogenous equilibrium. The experimental data required for calculation of the "biological value" are : (*a*) the daily wear and tear of nitrogenous tissue as measured by the nitrogen output in urine and faeces on a diet consisting of fat, carbohydrate and salts only, and containing no nitrogen from protein or other sources ; and (*b*) the minimum nitrogen intake required to balance exactly this daily loss, when the diet contains the protein under investigation as sole source of nitrogen.

The biological value of the protein is then equal to $\frac{a}{b}$.

The determination of (*a*) presents great technical difficulties, for nitrogen-free diets are extremely unappetising, and there is difficulty in securing adequate calorie intake over the period of time necessary to obtain trustworthy results. Experiments on themselves have been carried out by Thomas, (*Arch. Physiol.*, 1909, 219) and Martin and Robison (*J. Biochem.*, 1922, **16**, 407), and these observers agree in finding the daily excretion of nitrogen

due to wear and tear of body tissues equal to about 3·1 grms. N, or about 0·04 grm. N per kilo of body-weight. For the pig, the amount is 0·05 to 0·07 grm. N per kilo, and for the adult rat (300 to 400 grms. weight) 0·08 grm. N daily.

In the determination of (*b*) it is often difficult, and even impossible, to hit on the exact minimum, even after a long series of trials. Thomas suggested that if (*a*) be known, the biological value of a protein can be calculated from the *results of any one experiment*, whether the nitrogen balance be negative or even positive, (though in the latter case Thomas' argument is open to question). If, for example, the endogenous nitrogen excreted daily were 3·1 grms., and after daily administration of 7 grms. of nitrogen in the form of a particular protein, 8 grms. of nitrogen were excreted, it might be concluded that the 7 grms. of nitrogen ingested had sufficed to replace 2·1 grms. of the endogenous loss, and the biological value of the protein would $= \frac{2 \cdot 1}{7} = 0 \cdot 3$.

The following formula is applicable to the general case. If *au* and *af* = nitrogen excreted in urine and fæces, respectively, on a nitrogen-free diet, and if x = nitrogen intake, and y and z = nitrogen excreted in urine and fæces, respectively, in the experiment with protein P,

$x - (y + z)$ = nitrogen balance.

$z - af$ = nitrogen in the fæces from unabsorbed food, and

$x - (z - af)$ = true intake of nitrogen.

The biological value of protein P

$$\frac{\text{Body nitrogen replaced}}{\text{Food nitrogen absorbed}} = \frac{\text{Nitrogen balance} - (\text{balance on N-free diet})}{\text{True intake}}$$

$$= \frac{x - (y + z) + af + au}{x - (z - af)}$$

Using this calculation, Thomas arrived at the values given in Table II. below. Thomas' work has been criticised in that his separate experiments were of too short duration and that the intervals of time between them were not long enough for a wash-out

of residual nitrogen from the previous diet. Later investigators have come to the conclusion that Thomas' values are, in general, too high. Martin and Robison found the value of milk proteins to be 51, compared with Thomas' value of 100, and that of the proteins of whole wheat to be from 31 to 35, as compared with Thomas' value of 40 for those of wheat endosperm. As these workers point out, Thomas' formula assumes that the biological value of a protein remains constant when fed at different levels of physiological need. It has been shown experimentally, however, by McCollum in experiments on pigs, and by Mitchell (1924) with rats, that lower figures are obtained for the "biological value" of a protein as the proportion present in the diet is increased (see Table II.). It is essential in experiments of this type that adequate calories to cover all energy requirements should be supplied in the form of fats and carbohydrates, and Martin and Robison (*loc. cit.*) have shown that for calculation of biological values by Thomas' formula, the nitrogen balance should be determined for a nitrogen intake very near, but just below, the point of nitrogenous equilibrium. In their own work they made the required number of experiments to hit off this point as nearly as possible, and their figures are probably nearer the truth than those of Thomas.

The quantity of precise work that has been carried out on human beings is, however, small, and likely to remain so, as the experiments are tedious and require an extraordinary degree of sacrifice on the part of the subject.

The rat, being omnivorous in habits, is a suitable subject for such experiments. Mitchell (*J. Biol. Chem.*, 1924, **58**, 813) has used a method by which the nitrogenous metabolism of young growing rats can be accurately studied. He holds that the nitrogen excreted in the urine on a nitrogen-free diet represents the "endogenous nitrogen" derived from daily wear and tear of the tissues and remains constant, whatever the diet. His opinion that none of the faecal nitrogen is of endogenous origin is not, however, shared by other investigators. Mitchell holds, further, that the amount of *nitrogen retained* when any given protein is fed, represents the amount *required to repair this*

TABLE II

Relative biological value of proteins, as calculated from experimental determination of nitrogen balance

Protein.	Experimental animal.					
	Man.		Pig.		Rat.	
			Proportion protein in diet.		Proportion protein in diet.	
			Low.*	High.	5 per cent.	10 per cent.
Caseinogen	—	—	—	67‡	71	—
Zein	—	—	85*	55‡	—	—
Edestin	—	—	—	—	—	—
Gelatin	—	—	52*	—	—	—
Ox meat	100	—	—	—	92 to 97	68 to 84
Cow's milk	100	51	—	74‡	93	85
Fish	100	—	—	—	—	—
Rice	88	—	—	—	86	67
Potato	79	—	—	—	68	67
Pea	56	—	—	—	—	—
Wheat (whole grain)	—	35	—	45†	—	—
Wheat (endosperm)	40	—	—	—	—	—
Oats	—	—	—	47†	79	65
Maize	30	—	—	51†	72	60
Yeast	—	—	—	—	85	67
Observer	Thomas (*Arch. Physiol.*, 1909, 219)	Martin and Robison (*Biochem. J.*, 1922, **16**, 407)	McCollum (*Amer. J. Physiol.*, 1911, **29**, 215) McCollum (*Biol. Chem.*, 1914, **19**, 323), values calculated by Thomas' formula		Mitchell (*J. Biol. Chem.*, 1924, **58**, 905)	

* Nitrogen intake equal to the endogenous nitrogen (nitrogen excreted in urine on nitrogen-free diet).

† " " = 3 to 5 × endogenous nitrogen.

‡ " " = 9 to 11 × endogenous nitrogen.

daily loss of endogenous nitrogen, so that the biological value of the given protein

$$= \frac{\text{nitrogen retained}}{\text{nitrogen absorbed}}.$$

Using the same symbols as in dealing with Thomas' formula, *au* will = the "endogenous N."

The nitrogen retained from food in an experiment with protein P

$$= x - y - (z - af)$$

and the nitrogen absorbed

$$= x - (z - af)$$

Therefore biological value of protein P

$$= \frac{x - y - (z - af)}{x - (z - af)}$$

A series of values obtained by calculation based on this equation is given in the last column of Table II. Much more work on the subject will be needed to compose the divergence at present existing between the values obtained for the same protein by different observers.

In general, however, it is true that the foodstuffs investigated are ranged in the same order as regards relative biological value of their proteins, whether investigated by means of experiments on nitrogen metabolism or by observations on the growth of young animals.

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CHAPTER V

TANNINS

By C. Ainsworth Mitchell, M.A., D.Sc., F.I.C.

Official Method for Tanning Materials. *Qualitative Tests:* The Gold-beater's Skin Test Test for Phlobotannins Differentiation of Tanning Extracts Fluorescence Tests. The Constitution of Gallotannin. *Quantitative Methods:* Mitchell's Colorimetric Method Determination of Gallic Acid Analysis of Galls Myrobalans Catechol Tannins Osmium Tetroxide Method. *Gravimetric Methods:* Precipitation with Metallic Salts with Casein—with Alkaloids. Measurement of Colour of Tanning Solution.

Tannin analysis has been handicapped by our uncertainty as to the exact chemical constitution of gallotannin and other typical tannins. This uncertainty is reflected in some of the somewhat vague definitions of a tannin, which are often little more than lists of properties, the most distinctive of which is the capacity to "tan" hides. The measurement of their tanning power, irrespective of the chemical constitution of the tannins present, has thus become one of the most important of the methods for the commercial valuation of tan liquors and tanning materials for the leather industry, and much work has been done during the last few years to standardise the conditions under which the test should be applied if concordant results are to be obtained.

OFFICIAL METHOD FOR VEGETABLE TANNING MATERIALS

The method of using the hide-powder absorption process officially adopted by the International Society of Leather Chemists has been drawn up by Hugonin (*J. Soc. Leath. Trades Chem.*, 1926, **10**, 30). Standard conditions for the preparation of the

hide powder, the duration of mechanical agitation, and the filtration of the non-tans are specified.

As already indicated, absorption of a substance by hide powder is no absolute proof of its being a tannin, since non-tanning substances may also be absorbed. Thus it has been pointed out by Thuan and Favre (*J. Soc. Leath. Trades Chem.*, 1924, **8**, 346) that sulphite-cellulose extract is absorbed to a large extent, although it gives no precipitate with gelatin, and that sodium sulphoricinate behaves in the same way, and would thus be counted as a tannin. The use of nickel hydroxide, suggested by Singh and Ghose (*J. Soc. Chem. Ind.*, 1916, **35**, 159) as a substitute for hide powder, offers no advantage as an absorbent, and has the drawback that it absorbs gallic acid.

Reaction with Gelatin.—A. E. Jones (*Analyst*, 1927, **52**, 275) has shown that of seventy-three substances (non-tannins) tested, eighteen gave positive reactions with gelatin. These included: (1) In 1 per cent. solutions: gallic acid, β-resorcylic acid, hæmatoxylin, brazilin, picric acid, gallic acid, ethyl gallate, *m*-hydroxybenzoic acid, hydroxyhydroquinone, and maclurin. (2) Only in saturated solutions: phenol, resorcinol, catechol, phloroglucinol, methyl gallate, guaiacol, and protocatechuic acid.

Freudenberg (*Ber.*, 1920, **53**, 236; 1922, **55**, 1734, 1940) stated that catechin is precipitated by gelatin, but this has not been confirmed by Nierenstein (*J. Chem. Soc.*, 1922, **121**, 26; *Ber.*, 1922, **55**, 3832). The discrepancy appears to be due to the fact that impure catechin (or catechin which has been boiled for some time with water) gives a precipitate with gelatin, whereas the pure compound does not. Although Fischer's pentagalloyl glucose is precipitated by gelatin, it does not give a positive reaction in the gold-beater's skin test, and therefore does not behave like a true tannin (see p. 181).

QUALITATIVE TESTS

The Gold-beater's Skin Test. A qualitative test for tannins, based on their fixation on gold-beater's skin and subsequent staining with ferric chloride, was devised by Atkinson and

Hazleton (*Biochem. J.*, 1922, **16**, 516), and was subsequently developed and rendered more delicate by Price (*Analyst*, 1924, **49**, 25), whose modification is capable of detecting 0·00005 grm. of gallotannin in 1 c.c. of water. The technique of the test is as follows :

A small piece of gold-beater's skin (about $\frac{1}{2}$ inch × $\frac{3}{4}$ inch) is pinned on to a bed of paraffin wax contained in a watch glass.

Swelling.—One c.c. of 2 per cent. hydrochloric acid is pipetted on to the skin and left for ten minutes, after which the skin is washed for two minutes with water allowed to drop on to it at the rate of 2 drops per second.

Tanning. One c.c. of the solution under examination is left on the skin for thirty minutes, and the skin then washed as before for fifteen minutes.

Staining.—One c.c. of ferrous sulphate or ferrous chloride solution (1 per cent.) is allowed to remain on the skin for two minutes, after which the washing is repeated for two minutes as before.

Decolorising when Testing for Phlobaphenes.—One c.c. of 5 per cent. hydrochloric acid is left on the skin for five minutes, and the skin again washed, as before, for two minutes, and allowed to dry.

It may then be mounted for reference or comparison with similar pieces of the skin after treatment with gallotannin solutions of varying degrees of concentration.

Mitchell (*Analyst*, 1924, **49**, 29) has suggested the use of a device in which the skin fixed between an ordinary microscope slide and a perforated one, held together by means of screw clips, as being more simple ; it also enables the treated skin to be examined by transmitted light.

The sensitiveness of various reagents for tannin in this test was studied by Price, with the following results :

Ferrous sulphate and chloride were sensitive to 0·005 per cent. of gallotannin ; ferric alum and ferric sulphate to 0·01 per cent. ; ferric chloride to 0·05 per cent. ; ferrous ammonium sulphate to 0·1 per cent.

Mitchell's reagent (ferrous tartrate) was sensitive to 0·01 per cent. ; ammonium molybdate and vanadium chloride to 0·01 per cent. ; potassium chromate to 0·05 per cent. ; and copper sulphate to less than 0·1 per cent.

Mitchell (*loc. cit.*) found that osmium tetroxide is a useful

reagent for this test, but Price (*Analyst*, 1924, **49**, 336) has found that its sensitiveness is only about half that of ferrous sulphate.

The method has been studied by Jordan and Ware (*Pharm. J.*, 1924, **113**, 102), who find that it affords a valuable means of identifying and classifying drugs containing tannins. Extractives of certain drugs may stain the skin before the application of the iron salt, but, as a rule, it is possible to distinguish between the dyeing and the tanning effect. Stains due to dyeing are not usually enhanced by the addition of an iron reagent. All the tannin drugs in the B.P. Codex give a characteristic reaction in the test, with the exception of pyrethrum, which gives a doubtful result.

Iron and Ammonium Citrate Test. Ware (*Analyst*, 1924, **49**, 467) has found that, as a qualitative test for tannins, the iron and ammonium citrate of the British Pharmacopœia, used in association with ammonium acetate, is preferable to Mitchell's ferrous tartrate reagent.

Four c.c. of a clear aqueous extract are treated successively with 4 c.c. each of a solution of 0·25 grm. of iron and ammonium citrate in 100 c.c. of tap water, and of a 30 per cent. solution of ammonium acetate, and the mixture thoroughly shaken and boiled. The colour of the precipitate (if any) is noted, and the solution or filtrate is boiled with a little 10 per cent. ammonia, and a note taken of any further change. The precipitate should dissolve in dilute hydrochloric acid, but should be insoluble in dilute acetic acid or ammonia.

In testing a pharmaceutical preparation, whether aqueous or alcoholic, the sample is first diluted with 10 per cent. ammonium acetate solution, warmed and filtered, the precipitate washed with successive quantities of boiling water, to dissolve the "salted-out" tannin, the washings added to the filtrate, and the liquid tested as described above. This treatment removes most of the resins, fat, chlorophyll and other substances which might interfere with the test. Some of the less soluble phlobaphenes are also removed, but this is not a disadvantage, since they are not to be regarded as true tannins.

The results of the test may be summarised as follows:

(1) True tannins are completely precipitated. With gallotannins a purple precipitate is formed in the cold, and precipitation is complete on boiling. Without the ammonium acetate there is neither precipitation nor purple coloration.

(2) Hæmatoxylin gives a precipitate under the same conditions,

but may be distinguished from tannin by giving a deep blue solution, but not a purple precipitate, in the cold. On boiling with excess of the reagent there is a partial precipitation of a blue-black precipitate and a deep blue filtrate is obtained. A fresh decoction of logwood behaves similarly.

(3) Catechol, protocatechuic acid, catechins, pyrogallol, gallic acid and brazilin (decoction of sappan wood) all give purple-violet solutions on boiling, the colour changing to an intense red on adding ammonia. None of them gives a precipitate.

(4) Carbolic and salicylic acids, resorcinol, hydroquinone, phloroglucinol and anthraquinone give no characteristic result until ammonia is added, when the brown coloration is slightly intensified.

(5) Quercetin, rutin, aloin, and probably all other water-soluble anthroxanthin and oxymethyl-anthraquinone substances, give a brown coloration intensified by ammonia.

(6) Caffeotannic acid (so-called) and the tannin of hops are not precipitated.

Stiasny's Test for Phlobotannins. This has been studied by Ware (*Pharm. J.*, 1924, **113**, 104) in relation to various drugs containing tannin.

A few c.c. of the extractive are treated with a few drops of 40 per cent. formaldehyde solution, and afterwards with the same number of drops of 10 per cent. hydrochloric acid, and the mixture *boiled* for one minute (care being taken that there is an excess of formaldehyde and acid). After cooling and filtering, the precipitate is treated successively on the paper with water, 90 per cent. alcohol, and an aqueous solution of alkali, when a copious residue indicates phlobotannin.

The filtrate is treated with 1 or 2 drops of 10 per cent. ferrous sulphate solution, and then, drop by drop, with 5 per cent. potassium hydroxide solution. Unless the amount of chromogenic phenol is very small (or none) there is no precipitate of ferroso-ferric hydroxide.

Iron-greening filtrates are given by many anthroxanthin substances.

Iron-browning filtrates are given by certain substances containing anthraquinone derivatives.

Iron blueing filtrates are given by substances containing gallic acid, gallotannins and certain anthocyanins.

Classification of Tannins by Means of Tests with Iodine and Ammonia. Ware (*Pharm. J.*, 1924, **113**, 104) has devised a means of classifying tannins by the results obtained on boiling the drug or extract with a slight excess of an alcoholic solution of iodine. The following reactions may occur:

(1) Phlobotannin drugs give copious coloured precipitates, and the filtrate (in the absence of phenolic compounds) becomes practically colourless on the addition of ammonia. The precipitates formed by adding ammonia are either: (*a*) soluble, with formation of coloured solutions (e.g., iron-colouring substances not precipitated by Stiasny's reagent (*supra*), and substances which do not give a coloration with iron); (*b*) insoluble substances (which can be separated into iron-colouring and non-colouring); (*c*) partly soluble and partly insoluble.

(2) Phlobotannin substances containing gallic acid or gallotannin, or both, also give precipitates, but the filtrates give pronounced colorations on addition of ammonia.

(3) Substances containing only small quantities of tannin are characterised by reactions due to compounds other than tannins.

Differentiation of Tanning Extracts. A series of quantitative tests which enable different tanning material to be differentiated has been described by Jamet (*J. Soc. Leath. Trades Chem.*, 1922, **70**, 336). Among the tests less known are the following:

Nitrous Acid Test. An excess of sodium nitrite and 3 or 4 drops of 0·1*N* acid are added to a few c.c. of a cold 0·4 per cent. solution of the tannin. A coloration varying with the particular tannin indicates ellagitannic acid.

Stannous Chloride Test. Two c.c. of the tannin solution are treated with 10 c.c. of a strong solution of stannous chloride in hydrochloric acid. Mimosa and pine barks, notably larch, give a light rose colour.

Lead Acetate Test. Five c.c. of a neutral 10 per cent. solution of lead acetate are added to 5 c.c. of the tannin solution, the mixture filtered, and a portion of the filtrate treated with 10 per cent. sodium hydroxide solution (free from carbon dioxide). Mangrove, mimosa, oak bark, chestnut, myrobalans, valonia, divi-divi, algarobilla and gallotannic acid give no coloration. Quebracho gives a pale yellow; lentiscus and sumach a deep yellow; and wood pulp extract a yellow coloration.

Lauffmann's Test. The dry extract (2 grms.) is treated with 20 c.c. of strong potassium hydroxide solution in a silver crucible, and the mixture evaporated to a paste, which is then treated with dilute sulphuric acid, allowed to cool and shaken with ether. The extract is evaporated, the residue dissolved in about 5 c.c. of water, and the solution tested with a pine shaving.

Pine Shaving Test. A pine shaving is soaked in the tannin solution, allowed to dry, and then moistened with fuming hydrochloric acid. In the presence of phloroglucinol tans, such as gambier, a violet-red coloration is immediately obtained.

Wool-tinting Test. Extract corresponding in weight to 2 grms. of dry material is dissolved in 250 c.c. of water, and the solution heated for twelve to eighteen hours on the water bath with 3 grms. of white wool. After this time the wool is squeezed and transferred for fifteen minutes to a 1 per cent. solution of potassium dichromate at 70°, after which it is rinsed and dried. Sumac, nut galls and chestnut give a bright green shade. Mimosa, mangrove and quebracho give a brown coloration. Myrobalans give a yellow tint.

Differentiation of Oakwood and Chestnut Extracts (Stiasny). The filtrate from the lead acetate precipitation (*supra*) is treated with 10 drops of a 1 per cent. solution of ferric ammonium sulphate and about 0·5 grm. of crystalline sodium acetate.

Oak extract usually (but not invariably) gives no definite colour, whilst chestnut extract gives a blue-violet coloration.

Fluorescence Tests.—Meunier and Jamet (*J. Soc. Leath. Trades J.*, 1926, **10**, 166) have shown that certain tanning materials may be rapidly differentiated by their fluorescence when examined in ultra-violet light (Wood's lamp). Catechol tans (in acetone solution), for example, show a pronounced yellow fluorescence, whilst pyrogallol tans (chestnut, oakwood, myrobalans, divi-divi, and various galls) give a violet fluorescence. Mixtures of the two classes fluoresce bluish-white, and leaves which contain tannin (sumac, arbutus, lentiscus) and yield chlorophyll to the acetone show a dark red fluorescence.

THE CONSTITUTION OF GALLOTANNIN

Although the analyst who is examining tanning products for the specific purpose of making leather need not trouble about the exact nature of the tannin present, provided that it gives the

desired results, it is not possible to ignore its constitution in this way when a determination of the amount of tannin in such substances as tea or coffee is required. Hence a consideration of its chemical structure is an indispensable preliminary to the establishing of any analytical process of determining a tannin.

Chemical Formulæ for Tannin.—The formula worked out by Schiff (*Ann. Chem. Pharm.*, 1873, **162**, 43), according to which gallotannin is an anhydride of digallic acid—

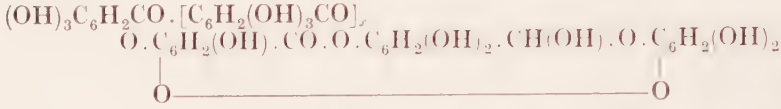
$$C_6H_2(OH)_3.CO{=}O(OH)_3H_2C_6,$$

was for many years accepted as correct, notwithstanding the fact that it was not in keeping with the observed molecular weight (above 600), as determined by the boiling-point method.

In 1908 Nierenstein (*Ber.*, **41**, 78, 3015) suggested a formula which agreed better with the optical properties recorded for gallotannin, although he did not accept the conclusion that glucose was an integral part of the tannin molecule. The view gradually gained support that even the purest preparations of gallotannin are not definite individual substances. For example, Iljin (*Ber.*, 1909, **42**, 1731), by fractional precipitation of gallotannin, separated a number of fractions which differed in composition and physical properties, and analogous results were obtained by Feist (*Arch. Pharm.*, 1912, **250**, 668; 1913, **251**, 468).

Meanwhile the revolutionary work of Fischer (*Ber.*, 1912, **45**, 922; 1914, **47**, 922; 1918, **51**, 1760; 1919, **52**, 829) on the synthesis of pentagalloyl glucose had apparently solved the problem, for his synthetic tannin behaved in many respects like the natural gallotannin. Nierenstein, however, pointed out (*J. Chem. Soc.*, 1921, **119**, 275) that there were several reasons against accepting Fischer's view (see also p. 184), the most important of these being the fact that, on methylating and hydrolysing the natural and synthetic tannins, different products were obtained; subsequently he put forward a "long chain" formula as being more in keeping with the observed facts. According to this later formula of Nierenstein (*J. Soc. Chem. Ind.*, 1922, **41**, 297)

gallotannin is a glucoside of a polyleuco-digalloyl digallic anhydride—



In attempting to obtain a standard gallotannin for his colorimetric method (*infra*), Mitchell discovered a remarkable product, derived from Chinese galls, which when hydrolysed yielded only a very slight amount of glucose (*Analyst*, 1923, **48**, 5). Nierenstein subsequently examined this specimen of gallotannin (*Analyst*, 1923, **48**, 321), and confirmed the fact that it was practically free from glucose.*

The results given by this remarkable tannin in colorimetric determinations agreed fairly well with Nierenstein's suggested formula (*supra*), but did not agree with the original formula of Schiff or with that of Fischer's synthetic pentagalloyl glucose.

From a survey, therefore, of the foregoing investigations it seems probable that the ordinary " pure " gallotannin or " tannic acid " is a mixture, in variable proportions, of different glucosides, mainly digalloyl glucoside, with a digallic anhydride of the type described by Nierenstein (*supra*). A mixture of approximately one-third of such a substituted glucose with about two-thirds of Nierenstein's anhydride would contain the requisite proportion of pyrogallic groupings (as shown by Mitchell's colorimetric method) and would yield the necessary amount of glucose.

* The later history of this tannin is curious. In 1924 Professor Freudenberg asked, as a favour, that some of it should be sent to him. This was done, but no acknowledgment was made, and nothing further was heard of it until last year, when Professor Freudenberg, in a lecture before the British Association, announced that a Dr. O. Schmidt had found it to contain 7 per cent. of glucose (" unpublished results "). It seems strange that it should have taken five years to find this glucose, and still more strange that the results should not have been published in the recognised scientific journals or communicated to those who had supplied the tannin. Until full details of these " unpublished results," as obtained at each stage of the determination, are published, as was done by Mitchell and by Nierenstein, this statement may be ignored, the more so since it is incompatible with the pyrogallol grouping in this tannin, the amount of which has been confirmed by several chemists working independently, and does not agree with the detailed examination of Hooper (see p. 194).

although the tannin from Chinese galls would require the presence of a small amount of one of the higher substituted glucoses to bring the molecular weight to the recorded value. This, however, would not affect the colorimetric factor (2·1) for Chinese galls, which is in accordance with the results obtained with such galls. Aleppo galls, on the other hand, yield a tannin which apparently consists of a different mixture of glucosides with a different anhydride, for its colorimetric pyrogallic ratio is 1·85.

It is thus obvious that no ordinary " pure " specimen of tannin can be accepted as sufficiently definite in constitution for it to be used, without correction, as a standard for establishing the accuracy of a colorimetric method. Even the " pure " specimen free from glucose, mentioned above, contained 10·5 per cent. of gallic acid, which had to be taken into consideration in the calculations. Prior to the introduction of Mitchell's colorimetric method there was no accurate method of determining gallic acid in the presence of gallotannin, and varying amounts of that tannin derivative must unquestionably have been present in the " pure " specimens used for standardising the results given by different gravimetric processes.

QUANTITATIVE METHODS

Mitchell's Colorimetric Method. The method devised by Mitchell (*Analyst*, 1923, **48**, 1) is based upon the principle that pyrogallol or the pyrogallic grouping in tannin combines with ferrous tartrate to form a violet compound the intensity of the colour of which is proportional to the quantity of pyrogallol.

The test is made by dissolving 0·1 grm. of the tannin, etc., in 100 c.c. of tap water, adding 2 c.c. of ferrous tartrate solution, and matching the resulting coloration with that produced by a solution of 0·1 grm. of gallic acid or pyrogallol in 100 c.c. of water.

The reagent consists of 0·1 grm. of ferrous sulphate and 0·5 grm. of Rochelle salt in 100 c.c. of water, and the coloration is con-

veniently matched in Nessler tubes provided with Hehner's side tubulures and taps.

The results may be expressed either in terms of pyrogallol, crystalline gallic acid or anhydrous gallic acid, for in each instance the pyrogallic group, $C_6H_2(OH)_3$, is the tinctogenic agent, and the water of crystallisation and the carboxyl group or (in the case of gallotannin) any glucose present merely serve to dilute that group.

Thus if the molecular weight of the substance under examination is compared with that of the standard, the ratios between the two will correspond with the proportions by weight of each required to give the same intensity of colour. For example :

	Molecular Weight.	Ratio.
Pyrogallol, $C_6H_2(OH)_3 . H$	125	1
Crystalline gallic acid, $C_6H_2(OH)_3 . COOH - H_2O$	188	1·50
Anhydrous gallic acid, $C_6H_2(OH)_3 . COOH$.	170	1·36

As was mentioned before, the method thus affords a means of deciding as to the correctness of the various formulæ which have been put forward for gallotannin.

For example, in Schiff's formula the proportion of pyrogallic groups is 78·1 per cent., and the ratio of pyrogallol to tannin would be as 1 : 1·28 ; in Fischer's synthetic pentadigalloyl glucose (molecular weight, 1,700·4) there are five pyrogallic groups, and the colorimetric ratio would be as 1 : 1·81. If in Nierenstein's polyleucodigalloyl digallic anhydride the term x is taken to represent unity, and the glucose is omitted, a compound with a molecular weight of 779 and containing two pyrogallic groups results, so that the colorimetric ratio would be as 250 : 779, or as 1 : 3·11 ; or if one molecule of glucose were added, the molecular weight would become 959 and the ratio as 1 : 3·8.

The ratio of the practically glucose-free tannin mentioned on p. 182 was as 1 : 3·32, after making allowance for the 10·5 per cent. of gallic acid and the 1·2 per cent. of moisture present.

Determination of Gallic Acid in Presence of Gallotannin.—For the colorimetric determination of gallic acid, gallotannin, gall

extracts, etc., the two substances are first colorimetrically determined together, the gallotannin then precipitated in a second portion, and the gallic acid again determined in the filtrate. For this purpose Mitchell (*loc. cit.*) found quinine hydrochloride to be the most suitable precipitant. The precipitated quinine tannate is washed several times with cold water (about 25 c.c. in all) and the filtrate and washings made up to 100 c.c. for the comparison.

Test experiments have shown that the method affords an accurate means of determining gallic acid in galls, myrobalans, tea, etc.

Analysis of Galls.—The older methods of determining the amount of tannin in galls by the hide-powder or oxidation methods gave misleading results, since the gallic acid is of as much value to the ink-maker as the gallotannin, and it is necessary to take into consideration the total amount of tinctogenic substances present.

Twelve average galls from a sample are crushed to a fine powder, which is thoroughly mixed. Five grms. of this powder are boiled, for an hour each time, with successive portions of about 150 c.c. of water, the extracts filtered, and the filtrate and washings made up to 500 c.c. Ten c.c. of this 1 per cent. extract are diluted to 100 c.c., and 1 c.c. of this 0·1 per cent. extract is used for the colorimetric determination of the total tinctogenic substances in terms of pyrogallol or gallic acid, and then of the gallic acid in the filtrate from the precipitated gallotannin. The difference between the two gives the gallotannin equivalent in terms of gallic acid. A series of empirical results with various samples of Chinese galls and Aleppo galls showed that the factor for converting the gallic acid equivalent of the former into gallotannin is 2·1, and for the latter 1·85. The moisture in the original galls and the total solids in the extract are also determined, and the sum of the results should be close to 100.

This method has thrown light on a number of interesting points. For example, in commerce it is commonly accepted that "white" galls (*i.e.*, those from which the larvæ have escaped) are less valuable than blue or green galls, which still contain the larvæ. From the point of view of the ink-maker, however, much will depend upon the degree of oxidation which has taken place within the galls. For example, the green galls in a commercial sample contained 61·8 per cent. of tannin and 2·5 per cent. of gallic acid, whilst the white galls in the same sample contained 52·9 per cent.

of tannin and 7·4 per cent. of gallic acid. The total tinctogenic values, however, were 23·9 and 24·0 respectively, so that whilst the tannin had decreased, the gallic acid had correspondingly increased, and the value of either to the ink manufacturer (though not to the tanner) was the same.

Roasted galls are a commercial product and are valued because they yield a darker ink. The effect of this process of heating to about 220° is to convert some of the gallotannin first into gallic acid and then into pyrogallol. Thus a sample of the green galls, mentioned above, was found, after being roasted, to contain only 37·0 per cent. of tannin, whereas the gallic acid and pyrogallol (in terms of gallic acid) amounted to 20·0 per cent., and the total tinctogenic value (in terms of pyrogallol) was 26·6. Hence, roasting the galls reduces the amounts of soluble extract and gallotannin, but greatly increases the proportion of non-tannin tinctogenic substances.

Myrobalans.—The yellow colour of the extract from such products as myrobalans or roasted galls interferes, to some extent, with the colorimetric method. As it is difficult to remove the colouring matter without also removing some of the tannin, the simplest plan is to make a correction by adding a trace of caramel or dyestuff to the standard solution, so that it matches the colour of the solution under examination, prior to the addition of the reagent.

Another plan is to attach a Lovibond tintometer glass of the right tint to the bottom of the Nessler tube by means of rubber bands.

For calculating the amount of tannin in myrobalans from the pyrogallol colorimetric equivalent it is necessary to take into consideration the fact that chebulinic acid, the tannin of myrobalans, has been shown by Freudenberg and Fick (*Ber.*, 1918, **52**, 1238; 1920, **53**, 1728) to be a crystalline compound of digalloyl glucose with a dibasic phenolic acid, $C_{11}H_{14}O_{11}$, which loses two molecules of water in the process of combining. This phenolic acid appears to contain a pyrogallic group, and a second pyrogallic group is present in the digalloyl part of the compound -

$$(C_6H_2(OH)_3.CO.O.C_6H_2(OH)_2.CO_2.C_6H_{11}O_6 + C_{11}H_{14}O_{11} - 2H_2O.$$

The molecular weight of this compound, $C_{34}H_{30}O_{23}$, is 806, and the colorimetric ratio between the two pyrogallic groups and the tannin is therefore as 1 : 3·22, or, in terms of gallic acid, as 1 : 2·14, which is very similar to that of the gallotannin from Chinese galls.

Error of Observation. Mitchell's colorimetric method has been tested by Nicholson and Rhind, and its limits of accuracy in the comparison determined (*Analyst*, 1924, **49**, 505). It was found that when the colorimetric comparisons were made in daylight the error of observation lay between — 2·59 and + 4·85 per cent. of the gallic acid present, but that better results could be obtained by the use of artificial light. With ordinary electric light, shaded by white paper, the errors ranged from — 1·81 to + 4·06 per cent., and with light blue paper shading they varied between — 4·31 and + 7·97 per cent.; by shading the light with dark blue crinkled paper, however, the errors were reduced to between — 1·33 and + 2·90 per cent., the average of seven readings being + 0·83 per cent.

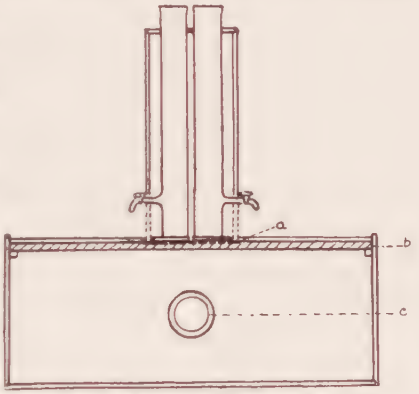


FIG. 8.—Nicholson and Rhind's colorimeter.

The apparatus shown in the diagram was designed to facilitate the making of the comparison. It consists essentially of an oblong box (18 × 7 × 7 inches), fitted with a plate-glass lid, and painted black inside: an electric bulb, C, is arranged as nearly as possible in the centre of this box. Above it is placed another box (5 × 7 × 10 inches), in which are placed the two Nessler tubes, with their taps projecting through slits in the sides. This box has no bottom, so that the Nessler tubes rest immediately over the electric bulb, with a screen of dark blue crinkled paper between the bottom of the tubes and the plate-glass. The rest of the glass lid is covered by movable wooden shutters. To eliminate errors of suggestion, the taps are connected by means of rubber tubing with a sink,

so that it cannot be seen how much solution is being run out from either tube.

Another modification introduced by Nicholson and Rhind is the addition of sodium chloride after precipitating the tannin with quinine hydrochloride, thus facilitating the filtration or centrifugal separation of the somewhat colloidal precipitate, the same amount of salt being added to the standard tube in each determination. Mitchell (*Analyst*, 1924, **49**, 509) had used Spanish clay for the purpose, thus avoiding the introduction of a soluble salt.

Catechol Tannins.—Price (*Analyst*, 1924, **49**, 361) attempted to extend the method to the determination of catechol tannins, but found that, although it was satisfactory for the comparison of (1) catechol with catechol, (2) protocatechuic acid with protocatechuic acid, and (3) catechin with catechin, yet the ratios obtained by comparing these three substances with one another were very unsatisfactory. Glasstone, however (*Analyst*, 1925, **50**, 49), showed that by a suitable adjustment of the *p*H value the method is also applicable to the catechol tannins, for the latitude permissible in hydrogen ion concentration varies with the particular substance. The range of *p*H limits observed were as follows :

	*p*H Limits.
Pyrogallol . . .	6·5 to 10·3
Gallic acid . . .	5·9 to 10·3
Gallotannin . . .	4·1 to 11·1
Catechol . . .	7·0 to 10·3
Protocatechuic acid .	6·3 to 10·4

Catechin produces its maximum coloration at a *p*H of 7 to 7·5, as does also protocatechuic acid, whereas, since catechol only gives its maximum at a larger *p*H value, the conditions must be adjusted before the two compounds can be compared.

Hence, when dealing with a substance for which the conditions for producing the maximum coloration are unknown, one of the following methods may be used : (1) Determine the *p*H value at which the violet colour is very faint, and work at about one unit above that value ; or (2) make up a series of mixtures of 1 c.c. of the solution of the phenolic body, 2 c.c. of the ferrous tartrate

reagent, and 5 c.c. of 10 per cent. ammonium acetate solution, and add different amounts of dilute ammonia (strength about 0·25*N*) to each mixture. If the ammonium acetate by itself produces an orange coloration, add dilute acid until the maximum violet coloration is obtained. Solutions thus prepared may be compared with each other independently of the respective hydrogen ion concentrations. This colorimetric method was used by Hooper (*Analyst*, 1925, **50**, 162) in studying the relative results obtained in comparative determinations of tannins by the hide-powder and cinchonine methods (*cf.* p. 194).

In studying the colorimetric behaviour of different substances with Mitchell's reagent, Price found that, to obtain the violet coloration with the ferrous tartrate reagent, it is necessary for the molecule to contain two hydroxyl groups in the *ortho* position. Thus phenol, hydroquinone, resorcinol, phloroglucinol, salicylic acid, meta-hydroxybenzoic acid, 3-resorcylic acid and guaiacol do not give the coloration, whereas catechol, protocatechuic acid, pyrogallol, gallotannin and gallic acid do give it.

Osmium Tetroxide Method.—It has been found by Mitchell (*Analyst*, 1924, **49**, 162) that osmium tetroxide gives a violet coloration both with compounds such as gallotannin, gallic acid and pyrogallol, with three adjacent hydroxyl groups in their molecule, and with catechol. It gives no coloration with phenol, salicylic acid, phloroglucinol, or resorcinol.

The colorations given by pyrogallol and catechol are similar, but the former reacts more rapidly than the latter. The initial reactions are apparently complete in about five minutes, and after about fifteen minutes the colour begins to darken, probably through absorption of oxygen. Hence, to obtain comparable results, it is essential to compare the colorations after completion of the five minutes' standing.

The molecular ratios between catechol (110) and pyrogallol (126) are as 1 : 1·145, and these ratios were obtained in actual determinations. Hence the entire substances appear to take part in the colour reaction, and the addition of one hydroxyl group to the catechol molecule increases its tinctogenic power by an amount corresponding to the increase in molecular weight. A similar

relationship can be established between the values for protocatechuic acid and catechol, the colorimetric ratio being 1 : 1·40, which is the ratio between the molecular weights. With gallic acid and gallotannin, however, it is not possible to establish a molecular relationship, and the colorimetric ratios of 1 : 1·1 found in each case must be regarded as empirical values.

The determination is made on similar lines to those used for the ferrous tartrate method (p. 183). The reagent consists of the ordinary 1 per cent. solution of " osmic acid " diluted with 10 parts of water, and a suitable standard for comparison is made by dissolving 0·1 grm. of pure pyrogallol, catechol, or gallic acid in 100 c.c. of water. One c.c. of this solution is added to 100 c.c. of tap water, and treated with 1 c.c. of the diluted reagent, and the resulting coloration is matched, after five minutes, with that given by 1 c.c. of a 0·1 per cent. solution of the unknown substance.

This colorimetric method has given satisfactory results in determining the tannin in tea, in sawdust, and in hops.

Hop Tannin.—Although hop tannin has not been isolated in a pure condition and its constitution has not been definitely determined, it has been shown by Chapman (*Analyst*, 1908, **33**, 95; 1909, **34**, 372) that the substance precipitated by cinchonine sulphate is probably the pure tannate (less colouring matter). Using this as the basis of comparison, Mitchell found that the factor for converting the colorimetric results into lupulo-tannin is 2·65.

Coffee Tannin.—Even the cinchonine gravimetric method has proved unsatisfactory for determining tannins in coffee (Smith, *Analyst*, 1913, **38**, 316), but the osmic tetroxide colorimetric method enables a determination to be made without difficulty. Results obtained after deduction of the gallic acid present gave 1·86 and 2·25 per cent. of tannins in Costa Rica and Nairobi coffee, respectively. It is interesting to note that by the use of this method it was possible to prove that part of the tannin in coffee is decomposed, on roasting, into products which no longer give a precipitate with quinine hydrochloride.

Tungstate Colorimetric Method. Menaul (*J. Agric. Res.*, 1923, **26**, 277) has described a colorimetric method for determining

tannin in plant tissues, based on the coloration given with a reagent prepared by boiling 100 grms. of sodium tungstate, 30 grms. of arsenious oxide, 300 c.c. of water, and 50 c.c. of hydrochloric acid for two hours beneath a reflux condenser and then diluting to a litre. The coloration given by this reagent with tannins is not affected by phenols, sugars or proteins, but reducing agents must not be present. For quantitative purposes the intensity of the coloration is matched with standard solutions containing 1 or 2 mgrms. of tannin in 50 c.c.

Obviously, the standardisation is, as usual, a weak point in this process, and a further drawback is that the reaction is not specific for tannins.

GRAVIMETRIC METHODS

Precipitation with Metallic Salts.—The earlier methods of determining tannin by precipitation as a metallic tannate have not proved satisfactory; firstly, because the composition of the tannate varies with the conditions of precipitation, and, secondly, because the tannates of metals adsorb substances other than tannin. Thus it has been shown by Mitchell (*Inks*, p. 90) that iron tannates can be precipitated in which the proportion of iron varies from 15 to about 22 per cent. If the iron tannate is precipitated in the presence of hydrogen peroxide a basic tannate, with 21 to 22·5 per cent. of iron, is obtained, but it has not been found possible to standardise the conditions so as to get concordant results.

The same criticism applies to the method of Ruoss (*Z. anal. Chem.*, 1902, **41**, 717), in which the tannate is precipitated by means of a solution of ferric sulphate in the presence of sodium tartrate (to prevent the formation of basic ferric oxide) and acetic acid (to dissolve the ferric hydroxide which would otherwise be simultaneously precipitated). I have been quite unable to obtain concordant results by this method, a very slight variation in the conditions causing the composition to vary from the 14·9 per cent. of iron required by a tannate of the formula $(C_{14}H_7O_9)Fe$.

Although it has been shown by Schoeller and Powell (*Analyst*, 1928, **53**, 517) that tannin can be used for the separation of earth

acids (tantalum and niobium oxides) from zirconia and hafnia, it is not practicable to use the converse process (precipitation of tannin by earth acids) for the determination of tannin, owing to the indefinite composition of the precipitates.

Gallic Acid.—Hirsch (*Chem. Ztg.*, 1927, **51**, 718) suggests the use of a 1 per cent. solution of bismuth nitrate (1·5 grms. in 30 c.c. of acetic acid, diluted to 240 c.c. with water) for the gravimetric determination of gallic acid. The yellow precipitate of subgallate is left to settle, collected on a weighed Gooch crucible, washed with hot water and dried at 45° to 50°, until constant in weight. When used with pure gallic acid the method gives results with an error ranging from −0·5 to −0·9 per cent. Obviously it cannot be used in the presence of tannin, and is thus of only limited applicability.

Precipitation with Casein.—It was shown by Nierenstein (*Chem. Ztg.*, 1911, **35**, 31) that tannin is precipitated by casein and may thus be separated from gallic acid or glucose, both of which are frequently found associated with tannin. Subsequently the method was modified and adapted to the determination of small amounts of tannin in fruit juices, etc., by Spiers (*J. Agric. Sci.*, 1914, **6**, 77) who, instead of determining the total solids in the solution before and after the precipitation, used a method of permanganate titrations, the difference between the two giving the equivalent of tannin, as was first suggested by Neubauer (*Z. anal. Chem.*, 1871, **9**, 1). The casein used was free from fat, and ammonium oxalate was used for standardising the permanganate, the tannin equivalent of the oxalate being taken as 0·4648 to 1 grm. This value was found experimentally as the mean of a number of determinations on different samples of "pure tannin."

The same principle has been applied more recently by Hartong (*Woch. Brau.*, 1929, **46**, 11) to the determination of tannin in worts and beers, the liquid being titrated with standard permanganate solution, in the presence of indigotin, before and after precipitation of the tannin with purified casein.

It was found in test experiments that, although the casein did not precipitate the whole of the tannin, yet, under definite conditions, it precipitated a constant proportion of it, so that the

amount of tannin originally present could be calculated from the difference between the titration results, on the assumption that 1 grm. equivalent of permanganate corresponds to 45 grms. of tannin, this relationship having been experimentally determined for hop tannin. The details of the method are as follows :

Two portions of 50 c.c. each of the wort or beer are treated with 1 grm. and 4 grms., respectively, of *caseinum purissimum* and shaken at intervals of five minutes during one hour. The liquids are then filtered, and 5 c.c. of each filtrate (which must be clear) are transferred to a 250-c.c. flask, treated with 20 c.c. of a dilute solution of indigotin, and titrated as rapidly as possible with *N* 100 permanganate solution until the colour of the liquid changes to a pale greenish-yellow. This titration is repeated four or five times and the average result is corrected by a titration of 20 c.c. of the indigotin solution alone. The corrected values thus obtained are deducted from the corresponding value obtained with the original sample before the treatment with casein. The difference is divided by 0·50 for the portion treated with 1 grm. of casein, and by 0·74 for the portion treated with 4 grms. of casein, since, under the conditions specified, the smaller amount of casein removes 50 per cent. and the larger 74 per cent. of the tannin originally present. The results, which are expressed in grms. of tannin per litre, agree within about 3 or 4 per cent.

A malt wort was thus found to contain 0·0111 per cent. of tannin, which was increased to 0·0191 per cent. after hopping, and the final beer after fermentation and storage contained 0·0152 per cent. Hartong concludes that probably the whole of the tannin in wort is present in combination with proteins.

The objections to this process, which is a development of the old Lowenthal method of determining tannin, are that it measures also substances other than tannin which are oxidised under the same conditions, and that the so-called "pure tannins" upon which the equivalent values have been based must have been substances of unknown constitution (see p. 183).

Precipitation with Alkaloids. It is commonly stated in the text-books that tannin is a general reagent for alkaloids, but it has been shown by C. M. Fear (*Analyst*, 1929, **54**, 316) that this commonly accepted view needs considerable modification. In experimental tests, in which various "pure" gallotannins were used, it was found that out of twenty-six alkaloids, including all

the commoner and a number of the rarer ones, only brucine, caffeine, cinchonine, cinchonidine and strychnine give appreciable precipitates. The relationship between the constitution of alkaloids and their precipitability has yet to be worked out.

Precipitation with Strychnine. Trotman and Hackford (*J. Soc., Chem. Ind.*, 1905, **24**, 1096) devised a method in which tannin was precipitated as strychnine tannate, but Spiers (*J. Agric. Sci.*, 1914, **6**, 77) found that the method was not accurate for gallotannin. The reason for this is probably that strychnine is much less readily precipitated than quinine or cinchonine by tannin.

Precipitation with Quinine. This gives quantitative results, and has been used by Tatlock and Thomson (*Analyst*, 1910, **25**, 103) for determining the tannin in tea, but the composition of the quinine tannates has not been so well studied as that of the cinchonine tannates.

Precipitation with Cinchonine. Chapman's method, as adapted to the determination of hop tannin (*J. Inst. Brew.*, 1907, **13**, 370; 1909, **15**, 360) was in the first instance standardised on a "pure" gallotannin which was assumed to be digallic anhydride. In view of the later work on gallotannin, however, it is doubtful whether the preparation had this composition; and apparently no allowance was made for any gallic acid present. Nevertheless, the method has proved trustworthy when checked by other methods, and has been used by Smith (*Analyst*, 1913, **38**, 312) for tea. In that case, however, it was found essential to remove the caffeine prior to the precipitation with cinchonine.

Hooper (*Analyst*, 1925, **50**, 162) studied the behaviour of cinchonine as a precipitant for tannin, checking his results by the hide-powder method and by comparison with Mitchell's colorimetric method. In one instance the following results were obtained: Colorimetric method, 77·8; hide-powder method, 76·8; cinchonine method, 76·8 per cent. Examining the glucose-free tannin discovered by Mitchell (see p. 182) he found that hide powder absorbed 84 to 85 per cent. of it, as against 87 per cent., calculated by difference from the analytical figures (water, 1·9; gallic acid, 11·1; diff. = 87 per cent.). The cinchonine tannate obtained from this tannin contained 3·9 per cent. of nitrogen,

corresponding to 40 per cent. of alkaloid ; the factor 0·6 thus gives 84 per cent. of tannin in the sample. These results are consistent with the absence of any material amount of glucose in this tannin (*cf.* p. 182). Hooper's general conclusion is that, although cinchonine is only a fairly good precipitant of tannin in certain materials, it may have a special value in determining tannin in the presence of catechin.

This, in fact, was proved to be the case by Adam (*Analyst*, 1928, **53**, 37) with cacao beans, in which the tannin and catechin were determined by a combination of the cinchonine method and Mitchell's colorimetric method. The results show that cacao catechin undergoes a change in the process of fermentation, and that no catechin is present in the fully fermented bean.

Jensen (*Analyst*, 1928, **53**, 365) has also used the method in studying the tannin in cacao beans. He found it essential in certain cases to extract the theobromine before precipitating the tannin with cinchonine. The tannate was collected on counterposed filter papers and dried at 105°. The nitrogen equivalent of the precipitates averaged 4·49 per cent., which is similar to the 4·43 per cent. found for the cinchonine tannates from hops and from tea.

A study of the precipitates given by quinine and cinchonine with gallotannin solution in excess has been made by A. E. Jones (*Analyst*, 1928, **53**, 429). He has found that cinchonine tannate, left in contact with an excess of a gallotannin solution, will adsorb about 4·8 times its weight of tannin. Hence it is not practicable to employ an indirect colorimetric method of determining certain alkaloids by adding an excess of tannin, and determining this excess colorimetrically in the filtrate from the precipitate.

Measurement of the Colour of Tannin Solutions. The Lovibond tintometer (see p. 85) is in general use for the measurement of the colour of tannin extracts, but has the drawback of being liable to a number of personal errors. A. de la Bruère (*J. Soc. Leath. Trades Chem.*, 1928, **12**, 485) has suggested the use of a photo-electric cell for the purpose. The rays from an iron arc lamp are made to act on the cell, which forms part of a circuit including a battery and galvanometer.

Successive readings are made with water and the solution under examination, with the use of special colour filters, and a curve is plotted in which the abscissæ are the colours arranged in spectroscopical order, and the ordinates are the deviations observed between the solution under examination and distilled water.

The principle used by Blackadder (*J. Soc. Leath. Trades Chem.*, 1923, **7**, 445) for measuring the colour of tanning solutions is the reverse of the Lovibond method. The instrument has a double field which is evenly illuminated, and the incident light passes through a variable opening in a rotating sector in the first half of the field, and through a cell containing the tannin solution to the second half. A half-light is used on the first half by means of a tint filter of 50 per cent. transmission, and the thickness of the solution under examination is varied until a match has been obtained. The lighter the colour of the solution, the greater the proportion of light transmitted and the higher the measurement.

The apparatus is of the Schmidt and Haensch type (*J. Amer. Leather Chem. Assoc.*, 1922, 209), in which the several colour measurements are made by taking observations through each of four colour filters in turn.

Procter (*J. Soc. Chem. Ind.*, 1923, **42**, 73) has devised a simple method which can be used with any colorimeter provided with three colour screens. These are calibrated by reference to standard flames, and the standard used is a 20 per cent. solution of iron ammonium alum, the numerical colour value of which is found by dividing the depth of the iron solution in mm. by the thickness (also in mm.) of the extract which it is required to match, when each colour screen (red, blue and green) is used.

The following example is given as an illustration :

A solution of gambier containing 0·42 per cent. of tannin required the following depths of iron solution to match it in a 14-mm. cell, with each of the screens : Red, 6·1 mm. ; green, 6·8 mm. ; and blue, 10·1 mm. The colour was, therefore, 6·1 ÷ 14 = 0·44 red ; 6·8 ÷ 14 = 0·49 green ; and 10·1 ÷ 14 = 0·78 blue. These figures were multiplied by 50·42 to obtain the colour of a 0·5 per cent. solution of the tannin.

Each colour value being less than unity, the solution was lighter in colour than the standard iron alum solution.

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CHAPTER VI

CEREALS

By D. W. Kent-Jones, Ph.D., B.Sc., F.I.C.

Introduction Wheat Rye Barley Oats Maize Rice General Properties of Flour for Bread-making Analytical Methods Moisture Ash Proteins Sugar Starch Colour Bleachers and Improvers.

Introduction.—Cereals, such as wheat, rye, barley, etc., and the flours therefrom are not substances which lend themselves easily to accurate analysis. Such analysis as is performed upon them is rather of an elementary nature, although of recent years, thanks mainly to American cereal chemists, more precise methods are being evolved. Analyses are, of course, made with the object of ascertaining or helping to ascertain the value of the material for its particular purpose. Obviously, therefore, where the cereal is one mainly used as a feeding stuff, the analysis is of an extremely empirical nature. On the other hand, with wheaten flour, analysis is undertaken with the object of advising the miller or the baker how best to utilise the material for such a complicated procedure as baking; for this, more detailed information is needed. This, together with the fact that wheaten flour constitutes the largest ingredient of civilised man's diet, has led to more precise knowledge and a wider range of analytical methods being employed.

WHEAT

Wheat can be grown over an extremely wide range of temperature and climate; hence the wheats used in commerce are grown in North America, South America, Australia, New Zealand, India, Manchuria, Japan, Persia, Egypt, East Africa, South Africa and in practically all of the European countries. Each and every kind of wheat has its own particular characteristics and peculiar

baking properties. It is impossible to correlate these absolutely with the various chemical characteristics as revealed by analysis, but there are certain broad features of distinction. It should be remembered that wheaten flour is used in a number of ways and that, although most of the flour is utilised for bread, flours are used for other purposes, such as for biscuits, cakes, pastries, etc. The desirable characteristics in flour for biscuit-making are, for

Wheat.	Moisture.	Average Ash.	Protein.
	Per cent.	Per cent.	Per cent.
No. 1 Manitoba	11·41	1·50	13·00
No. 2 Manitoba	12·19	1·50	12·54
No. 3 Manitoba	12·77	1·50	12·03
No. 4 Manitoba	13·38	1·60	12·26
No. 5 Manitoba	13·19	1·60	11·90
No. 6 Manitoba	13·53	1·70	11·90
Hard Spring	12·80	—	10·89
Hard Winter	12·35	—	11·20
Durum	13·07	—	11·40
Plate	11·50	1·80	11·50
Indian (Karachi)	10·00	1·85	9·50
Persian	10·50	2·30	10·50
Australian	10·93	1·40	8·5 to 12·0
Russian	12·16	—	11·90
German	16·00	—	9·50
French	16·00	—	8·00
English	16·00	1·60	9·50

example, very different from those required for bread-making. For biscuits, a soft flour of low protein content, which makes a rather unstable or even runny dough, is wanted. Such flours are called "weak." For bread-making, flours which have comparatively high protein contents are liked; these normally have high water absorption powers and produce stable, but still elastic or rubbery, doughs. Such flours are termed "strong." A strong wheat is broadly defined as one which will yield a flour capable of making large, well aerated and shapely loaves. Flour made from English

wheat, for instance, which is excellent for biscuit-making, has only poor water absorption and the resulting loaves are small and heavy.

The composition of wheat may be approximately given as: Starch, 63 to 71; proteins, 10 to 15; water, 8 to 17; cellulose, 2 to 3; fat, $1\frac{1}{2}$ to 2; sugars, $2\frac{1}{4}$ to $3\frac{1}{2}$; and mineral matter, $1\frac{1}{2}$ to 2 per cent.

Obviously, with the wide variation that is found in the plumpness of wheats, as shown, for instance, by the weight per bushel, there will be differences in composition.

The table on p. 199 gives typical results of the analysis of most of the better known and more widely used wheats of the world. It should be remembered, however, that wheats may vary in composition and baking properties from season to season.

CEREALS OTHER THAN WHEAT

For general analysis the methods given under wheat and flour will suffice for most cereals. The starches of the various cereals have distinctive forms when examined under the microscope.

Rye. Rye bread is eaten in eastern Europe. Rye from different districts has its own particular baking properties. In Europe it is generally admitted that Russian rye has the best water absorption and general baking property, but it has a poor colour. In Germany, therefore, it is usual to blend Russian and German rye. Ryes appear to have varied protein contents, but Stoa (*Agric. Exp. Sta. No. Dak. Agric. Coll. Bull.*, 1925, **193**) states that, on the whole, protein in rye is somewhat less than in wheat. The results in the table given at top of p. 201 were obtained by Kent-Jones (*Modern Cereal Chemistry*, 1927, p. 72) on 1926 samples of rye.

Similarly, rye flours, when examined by the usual procedure for estimating the distribution of the proteins, give widely different results. Rye flour is said to contain gliadin but no glutenin. Nevertheless, it contains a protein soluble in dilute alkali, and the amount of this can be estimated by the Blish-Sandstedt method, wherein the entire proteins are dissolved by alkaline methyl

ANALYSES OF RYES, 1926

	Moisture.	Nitrogen.	Protein (N × 5·7).
	Per cent.	Per cent.	Per cent.
Danubian	12·41	1·48	8·45
Russian	12·80	1·91	10·87
Plate	13·11	2·21	12·64
German	14·47	1·32	7·52
German	15·57	1·35	7·70
Chilian	11·28	1·76	10·04

alcohol and the protein corresponding to glutenin precipitated by acid at *p*H 6·4. Whether this is glutenin or not appears to be unknown. It is certain, however, that if it is, it will not form gluten with the gliadin present, as it is impossible to wash out gluten from rye flour. The results in the table were obtained by Kent-Jones (*Modern Cereal Chemistry*, 1927, p. 73) on rye flours sent in 1926:

ANALYSES OF RYE FLOURS

	Moisture.	Ash.	Total protein.	*p*H.	Acid *p*H.	Buffer.	Maltose.	Corrected to 13·5 per cent. moisture.		
								Protein sol. in H_2SO_4.	Protein sol. in alcohol.	Prot. in sol. in dilute alkali.
	Per cent.	Per cent.	Per cent.				Per cent.	Per cent.	Per cent.	Per cent.
English .	13·48	0·66	5·70	6·35	5·43	9·2	2·45	1·42	2·41	2·13
German .	14·30	0·75	6·52	6·25	5·03	12·2	1·92	1·15	2·88	2·81
,, .	13·08	1·13	7·40	6·05	5·17	8·8	1·64	1·50	3·11	3·57
,, .	13·96	1·32	8·30	6·23	5·35	8·8	1·59	1·84	3·11	4·03
,, .	14·50	0·40	3·74	6·03	4·85	11·8	1·54	0·75	1·79	1·67
,, .	12·64	0·58	5·20	6·00	4·67	13·3	1·71	0·46	2·53	2·20

Rye bread is dark and has a definite and sour flavour, which is much appreciated by some people. It is usually made with sour dough and not yeast. In Germany, however, it is becoming

customary to use 20 to 30 per cent. of wheaten flour with the rye, and this, of course, causes the dough to work less "dead" and produces a bigger and better aerated loaf. Such bread has a milder flavour.

Sometimes it is necessary to estimate the amount of rye flour which is present in a mixture of wheat and rye. König and Bartschat (*Analyst*, 1924, **49**, 187) suggest that the best method is by the estimation of the percentage of the total protein which is soluble in calcium sulphate. Ten grms. of the flour are moistened in a 500-c.c. flask with saturated calcium sulphate solution at room temperature (0·22 grm. in 100 c.c.), made up to the mark and shaken for one hour. The nitrogen in 100 c.c. is estimated, and this is expressed as a percentage of the total nitrogen in the flour. Wheat shows 29·1 per cent. and rye 51·5 per cent. Mixtures can be interpolated thus :

Wheat.	Rye.	Per cent.
100	—	29·10
90	10	31·34
80	20	33·58
70	30	35·82
60	40	38·06
50	50	40·30
40	60	42·54
30	70	44·78
20	80	47·02
10	90	49·26
—	100	51·50

The experimental error is about 5 per cent., but this may be greater with acid flour. Strohecker (*Analyst*, 1924, **49**, 282) also suggests methods based on the refractive index and conductivity of the water extract.

Barley.—Barley is of importance in view of its great use in brewing. For this it should be even in size, dry and free from damaged corn. It should be plump and, when cut across, show a

loose, white, mealy surface. The following table gives the composition of various kinds of barley :

COMPOSITION OF BARLEY

	English barley (Warrington).	German barley (Kellner)			Feeding.	American barley (Wiley).
		Medium ground.	Large ground.	Flat ground.		
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water . . .	14·3	14·3	14·3	14·3	14·3	10·85
Proteins . .	10·6	9·4	8·7	10·2	12·0	11·00
Fat . . .	2·1	2·1	1·8	2·5	2·4	2·25
Soluble carbohydrates . .	66·0	67·8	70·2	63·7	63·7	69·55
Fibre . . .	4·5	3·9	2·7	5·0	5·0	3·85
Ash . . .	2·5	2·5	2·3	2·8	2·6	2·50

Osborne (*The Vegetable Proteins*, 2nd Ed., 1924) gives the following as the proteins of barley : Leucosin, 0·30 ; hordein (a prolamin soluble in dilute alcohol), 4·0 ; edestin (a globulin, *i.e.*, insoluble in water, but soluble in dilute salt solution) ; proteose, 1·95 ; and insoluble proteins, 4·5 per cent. Barley, like other cereals, contains soluble sugars (chiefly sucrose, but also dextrose, raffinose, etc.), and these may amount to as much as 3·4 per cent.

Although over half the consumption of barley in the United Kingdom is for brewing purposes, yet a considerable proportion is used for cattle feeding. Wood (*Products of Barley*, p. 248 ; *Farm Crops*, I., 1925) gives the following average composition of barley used for feeding :

Protein, 11·0 ; fat, 1·5 ; carbohydrate, 66·5 ; fibre, 4·5 ; ash, 2·5 ; and water, 14·0 per cent.

Oats.—This cereal is another important crop. For milling into oatmeal tough, plump and thin-skinned oats, rich in protein, are preferred. Berry (*Products of Oats*, pp. 188–197 ; *Farm Crops*,

I., 1925) gives the following analyses of the component parts of oats :

ANALYSES OF COMPONENT PARTS OF OATS

	Oat			Wheat.
	Kernel.	Husk.	Grain.	
	Per cent.	Per cent.	Per cent.	Per cent.
Moisture . . .	13·40	6·77	13·40	13·4
Protein . . .	12·34	2·45	9·46	12·1
Oil	7·73	1·27	5·33	1·9
Carbohydrates . .	63·47	52·20	60·23	69·0
Fibre . . .	1·33	33·45	8·96	1·9
Ash	1·83	3·86	2·62	1·7

Maize.—This is in some respects the world's most important cereal, as it is probably the cheapest food available for mankind over a large part of the civilised and partly civilised world. Besides being used as a human foodstuff, it is in demand for the manufacture of starch and cellulose ; it is also, of course, a popular cattle food. The principal protein in maize is the prolamin, zein, which normally constitutes half the total protein. The following table gives the chemical composition of maize :

CHEMICAL COMPOSITION OF MAIZE

	Hull.	Embryo.	Endosperm.
	Per cent.	Per cent.	Per cent.
Protein	6·6	21·7	12·2
Ash	1·3	11·1	0·7
Fat	1·6	29·6	1·5
Carbohydrates, nitrogen-free extract	74·1	34·7	85·0
Crude fibre . . .	16·4	2·9	0·6

Rice.—The rice-eating nations, as they are called, are known to all, although it is interesting to note that many of these countries are gradually changing over to wheat. Rice containing husk is

Analytical Data on Rice

	Percentage Organic Composition.						Total Mineral Constituents.					
	Moisture.	Proteins.	Fat.	Carbohydrate.	Fibre.	Ash.	N_2.	SiO_2.	CaO.	MgO.	P_2O_5.	K_2O.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Unhusked rice . .	12·55	6·35	2·14	65·19	7·84	5·93	0·92	3·84	0·04	0·01	0·54	—
Husked rice . .	11·68	7·71	1·19	77·79	0·70	0·93	—	—	—	—	—	—
Skinned rice . .	13·02	6·91	2·24	75·71	0·68	1·44	1·11	0·13	0·03	0·19	0·69	0·30
Polished rice . .	12·90	6·47	0·46	79·43	0·25	0·49	1·01	0·02	0·02	0·05	0·24	0·08
Meal . . .	8·10	11·50	13·50	53·50	4·50	8·90	1·88	1·40	0·06	0·99	3·86	1·46
Polish . . .	11·40	10·20	7·80	63·20	1·20	6·20	1·64	0·97	0·04	0·18	2·82	—
Dust . . .	9·60	6·20	3·80	41·00	22·10	17·30	1·13	11·50	0·07	0·45	1·09	—
Husks . . .	—	—	—	—	—	—	0·03	17·18	—	—	0·04	—

known as paddy. Paddy yields about five-eighths of its weight of cleaned rice. The world produces approximately 100,000,000 tons of cleaned rice annually.

The most important protein in rice is oryzenin, a glutelin. It is present to the extent of about 7 per cent., and as a glutelin it is soluble in dilute alkali.

The table on p. 205 which is taken from the *Imperial Institute Bulletin* (1917) gives some analytical data on rice.

GENERAL PROPERTIES OF FLOUR FOR BREAD-MAKING PURPOSES

There are two broad essentials for suitable bread-making flours :

(1) There must be sufficient sugars present and sufficient diastatic activity to produce sugars (as those originally present are used up by the yeast), so that there is always enough gas to distend the dough well.

(2) The proteins of the dough must be sufficient in quantity and good enough in quality to hold the gas.

Originally the importance of the first requirement was not so well recognised, as Jago (*Technology of Breadmaking*, 1911, p. 335) indicated that flours contained, or formed readily in the cold with water, sufficient sugars for the production of all the gas necessary in the normal fermentation of dough. Wood (*J. Agric. Sci.*, 1907, **2**, 139), however, had suggested that strength should be separated into two factors, namely, those of volume and shape. He suggested that the capacity of a flour for giving off gas when incubated with yeast and water is the factor which primarily determines the size of the loaf.

More attention, however, was paid in the early days to the second requirement. Beccari, in 1745, had first placed on record the fact that gluten could be washed out of flour, and in the middle of the nineteenth century a number of investigators were beginning to associate strength with the amount of gluten. Later, it was noticed that the quality of the gluten, or what would be now termed the particular colloidal state of the gluten, was of as much

if not more, importance than the quantity. It was soon found that the gluten estimation left much to be desired, owing to the failure of chemists to get results which were in any way comparable. Standard methods of washing, the use of special washing-out solutions, etc., were all recommended at various times. In this connection mention should be made of Bénard and Giradin (*J. Pharm. Chim.*, 1881, [5], **4**, 127), Ballard (*Ann. Chim. Phys.*, 1884, [6], **1**, 533; *Compt. rend.*, 1893, **116**, 202), and particularly of Arpin (*Ann. chim. anal. appl.*, 1902, **7**, 325, 376, 416). Fleurent (*Compt. rend.*, 1905, **140**, 99) recommended the use of a solution containing 0·1 grm. of calcium carbonate per litre for washing-out. It was soon noted, however, that gluten was not pure protein (Norton, *J. Amer. Chem. Soc.*, 1906, **28**, 8), but that it also contained fat, starch, mineral matter, fibre, etc. Osborne (*Amer. Chem. J.*, 1893, **15**, 392) showed that the two main protein constituents of wheat flour were gliadin, a prolamin soluble in 70 per cent. alcohol, and glutenin, a glutelin soluble in dilute alkali, but that there were also present smaller quantities of an albumin, a globulin and a proteose. This discovery suggested to a number of workers that the ratio of gliadin to glutenin in flours might vary and be related to the baking quality. This was investigated particularly by Fleurent in France (*Compt. rend.*, 1896, **123**, 327, 755; 1898, **126**, 1374, 1592; 1905, **140**, 99) and Snyder in America (*Minn. Agr. Exp. Sta. Bulls.*, 54, 62, 63; *U.S. Dept. Agr. Off. Exp. Sta. Bull.*, 104). Owing to the fact, however, that the methods employed are now known to have been unsatisfactory, the results are not of great value.

ANALYSIS

For ordinary purposes the following determinations are required for a complete analysis of flour: Moisture, ash, protein, gluten, sugar (as maltose), fat, starch and fibre.

Generally the first five suffice. In addition to these, methods attempting to measure colour are employed, whilst sometimes determinations of the hydrogen ion concentration, titrable acidity, etc., are undertaken. Wheat and other whole-grain analysis is

usually restricted to the determination of the weight per bushel, moisture, amount of foreign seeds and nitrogen content. None of these calls for special comment as regards recent advances, save the determination of nitrogen, which is carried out as is described later under the paragraph on flour. It is, however, of use to record the factor converting weight in pounds per bushel in kilos. per hectolitre:

Weight in pounds per bushel × 1·248 = kilos. per hectolitre.

Kilos. per hectolitre × 0·8012 = pounds per bushel.

For the accurate determination of the weight per bushel, the Louis Schopper 20-litre balance is employed, but it is a costly installation. Small laboratory weight-per-bushel apparatus are often inaccurate and are really only helpful for comparative purposes.

Moisture.—Moisture in cereal products has obviously different degrees of combination. There is ordinary moisture and also the more closely associated moisture, possibly colloidally attached, which may be termed "moisture of constitution." While there have been numerous methods employed for the determination of moisture in cereals and cereal products, the most reliable generally are those based on the loss of weight occurring on heating in an oven. Distillation methods with oil have been used, but these have to be very carefully standardised. While the Brown-Duval, a distillation method, is still the official method in U.S.A. for the moisture in wheat and other grains (not flour), these methods have not so far found popularity in the United Kingdom. For the successful employment of the Brown-Duval method, it is essential to follow out the empirical conditions laid down exactly as to the height of the burner, time of heating, size of sample, etc.

Where the method relies on the loss of weight during heating, the result depends mainly on the kind of oven and the temperature employed. For instance, the legal limit of moisture in flour in U.S.A. was fixed at 13·50 per cent., as determined by heating in a water oven. Recently, the standard has been raised to 15·00 per cent., as determined in a vacuum air oven. The following quotation will make it clear that 13·50 per cent. determined in a water

oven is considered the same moisture as 15·00 per cent. determined in the vacuum oven.

"Flour may contain not more than 15 per cent. (15·00%) of moisture as determined by the vacuum method of the Association of Official Agricultural Chemists in accordance with a revised and amended definition and standard adopted, upon the recommendation of the Food Standards Committee, by the Secretary of Agriculture for the guidance of officials in the enforcement of the Federal Food and Drugs Act.

"The change in the standard is not, in the opinion of the Foods Standards Committee, an actual increase in the moisture permitted in flour, since the water-oven method previously used to determine moisture did not give all of the water present within about 1½ per cent. (1·5%). It has been found by careful experimental work that 13½ per cent. (13·5%) by the water-oven method, the standard formerly enforced, is equivalent to 15 per cent. (15·00%) by the new method. The change in this standard is an official recognition of a more accurate method for moisture determination in flour rather than any change in the amount of moisture permitted in flour."

Numerous investigators have pointed out the difficulty of operators getting concordant moisture results. Leatherock (*J. Amer. Assoc. Cer. Chem.*, 1922, **7**, 102) sent sixty-nine sealed samples of the same flour to as many different and skilled operators. The average result was 14·19 per cent., distributed between 13·22 and 15·00 per cent. The average of all fifty-nine air-oven results was 14·15 per cent., ranging from 13·22 to 15·00 per cent. The average of all vacuum oven results was 14·45 per cent., ranging from 14·10 per cent. to 14·74 per cent. Thus it will be noticed that the vacuum ovens gave an average result on the sample of 0·3 of 1 per cent. higher. The more extreme variations occurred with the air ovens, there being a difference of 1·78 per cent. between the highest and lowest of these, whilst the corresponding difference with the vacuum ovens was only 0·64 per cent.

Eight operators, using air ovens and samples of 1 to 4 grms., obtained results averaging 13·97 per cent.; low, 13·22 per cent.; high, 14·47 per cent.

Twenty-nine operators, using air ovens and 5-grm. samples, obtained an average of 14·22 per cent.; low, 13·36 per cent.; high, 14·60 per cent.

Twenty-two operators, using air ovens and 10-grm. or larger samples, obtained an average of 14·15 per cent.; low, 13·58 per cent.; high, 15·00 per cent.

Six operators, using regular Freas vacuum ovens, obtained an average of 14·48 per cent.; low, 14·20 per cent.; high, 14·74 per cent.

Three operators, using Mojonnier ovens, obtained an average of 14·49 per cent.; low, 14·20 per cent.; high, 14·64 per cent.

One operator, using the latest model Mojonnier oven, secured a result of 14·10 per cent.

Leatherock's main conclusions were that vacuum ovens and air ovens cannot be used to check each other, and that with all ovens the temperature employed, the time of drying, etc., should be standardised within close limits. He advises the taking of "grab" samples instead of weighing out a definite amount, and even the standardisation (of shape, size, etc.) of the container used for storing the samples. Nelson (*J. Amer. Assoc. Cer. Chem.*, 1923, **8**, 171) also investigated flour moisture and pointed out that flour, when dried, is even more hygroscopic than calcium chloride. Dried samples should, therefore, be cooled and weighed in containers with the lids on. He stated that the moisture colloidally attached can only be driven off at high temperatures, and that the speed of drying in vacuum ovens is largely influenced by the number of samples in the oven, that is, by the total amount of flour being dried.

It is certainly sound practice in determining moisture in cereals to place all the samples in the oven at the same time and not to open the door until the drying operation is over.

Snyder and Sullivan (*J. Ind. Eng. Chem.*, 1924, 741, 1163; 1925, 311; 1926, 272) critically examined various factors in moisture determinations. They concluded that the main quantity of the free moisture is driven off in water-oven drying, and that much of the remaining moisture, expelled at higher temperatures, is in a different form.

Any form of drying oven, operated with care, and with always exactly the same procedure, should given consistent and useful results. Such results, however, are only comparable with others

obtained under exactly the same conditions. In view of the fact that many drying ovens vary in heat intensity from place to place, it is advisable to test the oven every few weeks by putting in samples from one and the same flour in different parts of the oven at the same time, and seeing that the results are in close agreement.

Recently Coleman and Dixon (*Cer. Chem.*, 1926, **3**, 419) examined a quick moisture tester for cereal products which has since been placed on the market under the name of the Carter Simon Oven. The oven is small, measuring 8½ inches long, 7½ inches wide and 7½ inches high. The actual heating chamber is 2 inches high, 3 inches wide, and runs the length of the oven. It is provided with closely fitting, asbestos-insulated, hinged doors on each end of the oven. These doors operate simultaneously and provide for the insertion and removal of the moisture tins. Three moisture-testing tins can be placed in the heating chamber at one time. The moisture tins are of aluminium, and are 2½ inches in diameter and ¾ inch in height. The tins are also provided with closely fitted lids for use during cooling and weighing. The oven is heated by electricity. Temperature is controlled by means of a thermo-regulator, which is suspended through the top of the oven into the heating chamber.

Ventilation is obtained by means of two series of holes, ¼ inch in diameter, and a metal stack, 9 inches high and 1 inch in diameter. Air enters the oven through a series of holes located on the outside about ½ inch from the sides of the door at the stack end of the heating chamber. The second series of holes is located at the opposite end of the oven within the heating chamber itself. At this point the outside air, which has been previously heated, enters the heating chamber. The movement of the air is accomplished by means of the 9-inch stack previously mentioned, which is located on that end of the oven at which the outside air enters. The air thus circulates in a clockwise direction, as it enters and leaves the moisture oven.

Five-grm. samples are weighed out into the tins, and it is essential that the finely ground material be spread very evenly over the bottom of the tin. The oven is operated at 155° C., but an alteration of a few degrees makes very little difference. The

material is weighed out in triplicate, and each tin is inserted exactly five minutes after the previous one. Every tin thus successively occupies each position in the oven, and the result is given by the average of the triplicate readings the usual precautions in the use of the desiccator, etc., being observed. Moisture results can, therefore, be carried out in fifteen minutes. The drawback is, of course, that the oven wants continual attention.

The table on p. 213 gives results obtained by Coleman and Dixon with this oven, compared against heating at 108° C. for five hours and at 130° C. for one hour (the latter being the tentative method adopted by the Association of Official Agricultural Chemists as a substitute for the vacuum oven test).

Ash.—Considerable attention has been paid of recent years to the accurate determination of the ash content of cereals. Although this determination is not difficult, it is rather lengthy, and many attempts have been made to shorten the time required. It is obvious that neither too high nor too low a temperature should be employed, and that the ash should be as white and free from carbon as possible. It is, therefore, generally agreed that fusing of the ash should be avoided, but, if this only occurs towards the end of the operation, the error is very slight. The ash of wheat flour fuses at about 700° C., and much above this temperature volatilisation of some of the salts may occur. A convenient method is the use of an electric muffle furnace operated at 600° C. Below 550° C. the time taken is considerably longer than the one and half to two hours necessary at the suggested temperature, whilst, if the time is shortened, the results may tend to be high. Five-grm. samples are normally employed for flour and ground-up grains generally, the container being either a flat platinum or silica dish ($6\frac{1}{2} \times 3\frac{1}{2} \times 1$ cm. is a convenient size). Gold with 5 per cent. of platinum is a convenient material for these dishes. With bran and suchlike materials of a bulkier nature and high ash, smaller quantities should be used. It is convenient to have a small chimney (18 inches high, for example) at the end of the oven, and for the door to have a small round hole ($\frac{1}{2}$ inch in diameter) which can be opened or closed by a swinging shutter. It should be closed for the first fifteen minutes or so until the preliminary combustion

is over and then adjusted to hasten the burning away of the carbon cake. There is practically no difference in weight between a white ash and one light grey in colour. When an excess of

COMPARISON OF THREE METHODS FOR MAKING MOISTURE TESTS ON FLOUR (COLEMAN AND DIXON, 1926)

Sample No.	Moisture content of flour.			Difference from A.O.A.C. method.	
	Five hours at 108° C.	Over night in 130° C. oven.	Fifteen minutes at 135° C.*	Fifteen minutes' method.	108° C. method.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1	12·51	12·75	12·69	− 0·06	− 0·24
2	12·29	12·56	12·51	− 0·05	− 0·27
3	13·15	13·36	13·38	+ 0·02	− 0·21
4	12·68	12·79	12·73	− 0·06	− 0·11
5	12·62	12·85	12·84	− 0·01	− 0·23
6	12·76	12·97	12·84	− 0·13	− 0·21
7	12·04	12·30	12·24	− 0·06	− 0·26
8	12·50	12·75	12·66	− 0·09	− 0·25
9	12·43	12·69	12·68	− 0·01	− 0·26
10	12·12	12·34	12·20	− 0·14	− 0·22
11	12·06	12·34	12·30	− 0·04	− 0·28
12	12·48	12·82	12·78	− 0·04	− 0·34
13	11·83	12·06	12·04	− 0·02	− 0·23
14	12·46	12·61	12·66	+ 0·05	− 0·15
15	12·24	12·46	12·42	− 0·04	− 0·22
16	11·97	12·18	12·16	− 0·02	− 0·21
17	11·82	12·26	12·27	+ 0·01	− 0·44
18	12·44	12·69	12·69	0·00	− 0·25
19	12·18	12·44	12·43	− 0·01	− 0·26
20	12·26	12·52	12·49	− 0·03	− 0·26
21	13·58	13·86	13·84	− 0·02	− 0·28
22	11·78	11·98	12·00	+ 0·02	− 0·20
23	13·18	13·38	13·32	− 0·06	− 0·20
24	9·12	9·36	9·30	− 0·06	− 0·24
25	8·78	9·04	9·00	− 0·04	− 0·26

* The latest type of this oven is operated at 155° C.

chlorides is present (as, for example, in bread) it is best to char at a low temperature, *e.g.*, over an Argand burner. The char is then well extracted with water and filtered. The bright filtrate is evaporated to dryness in a bowl and finally dried in a water oven.

The wet char and the paper are then incinerated, and the weight of the ash obtained is added to the weight of the dried residue from the extract. The use of glycerin and alcohol, hydrogen peroxide, acetate, etc., has been advocated in U.S.A. from time to time to accelerate the test, especially when low temperatures, such as 500° C., are used, as is normal in that country. Of these suggestions, the use of glycerin and alcohol seems to have gained the most popularity. The method suggested by the American Association of Cereal Chemists, for instance, is as follows :

" Rapidly weigh 3 to 5 grms. of the well-mixed sample into a shallow, relatively broad ashing dish which has been ignited, cooled in a desiccator, and weighed soon after reaching room temperature. Add 1·5 c.c. of glycerol-alcohol solution (1 : 1 : if a technical grade of glycerin is used, a blank determination is necessary) to the ashing dish for each grm. of the sample to be ashed. Allow the glycerol-alcohol solution to soak into the sample for at least five minutes. Ignite the alcohol, and then place the crucibles in an ashing furnace which has been heated to a dull red colour or to a temperature not in excess of 600° C. (1,112° F.). Incinerate at this temperature until the ash becomes fluffy and white or grey-white in colour, or until no further loss in weight occurs. Cool in a desiccator and weigh soon after the crucibles have reached room temperature. Calculate to per cent. of ash. Re-ignited calcium oxide is a suitable desiccating agent."

Another quick method is the oxygen acetate test advocated by Brendel and used in some of the American mills :

" A charge of the sample to be ashed is weighed up in a platinum crucible (low form most desirable), and allowed to char into a cinder in the muffle. Removing the crucibles after burning contents to a cinder, 2 c.c. of a calcium acetate solution, the strength of which is approximately 60 mg. to 1 c.c., is pipetted into the crucible, completely covering the cinder while yet quite warm. The acetate solution is allowed to evaporate by placing the crucibles on the opened muffle door. When the cinder is completely dried, the crucibles are placed in the muffle, the temperature of which is carried at practically 1,500° F. After the samples have been in the furnace three to four minutes, oxygen is delivered into the muffle for a period of ten to fifteen minutes. The length of time that the oxygen is sent into the furnace depends largely upon the number of samples, temperature used, and the pressure of oxygen current. The period of time required for ashing a sample or samples can be regulated to meet the chemist's convenience. The ash

resulting from this method is of a very light, fluffy white character. When the 'glow' around the bottom of the crucibles disappears, it is an indication that the ash is 'down.' A blank determination is made, using 2 c.c. of the calcium acetate solution, and correction made for the acetate used. Calcium oxide is used for desiccation. As platinum crucibles cool very rapidly, the time required for cooling is much shorter than when other types of crucibles are used. The balance work should be rapid in using this method."

An analysis of the ash of wheat flour is not usually required. The calcium may be determined either in the ash or in the flour itself after the starch has been saccharified. Care must be taken to avoid contamination by the magnesium (Kent-Jones, *Modern Cereal Chemistry*, 1927, p. 341). The natural sulphate in cereal flours or meals can also be determined in the clear solution obtained by saccharifying the starch and filtering off the proteins in the cold after standing some time. The sulphate, natural to flour, cannot be found in the ash, as the excess of acid phosphate drives it off during the incineration. The percentage composition of the ash of English wheat is approximately (average of several samples) :—

	Per cent.
Phosphoric anhydride, P_2O_5	53·20
Potash, K_2O	29·97
Magnesia, MgO	11·76
Lime, CaO	2·56
Iron oxide, Fe_2O_3	0·79
Silica, SiO_2	0·72
Sulphuric anhydride, SO_3	0·51
Soda, Na_2O	0·45
	99·96

The table on p. 216, given by Teller (*Ark. Agr. Exp. Sta. Bull.*, 1896, 42, Part II.), shows that the proportion of potash and lime increases as the grade of flour improves, but, on the other hand, the proportion of magnesia decreases from the bran to the patent flour. This had also been shown by Dempwolf (*Chem. Pharm.*, 1869, **149**, 343).

Protein. The analysis of cereal products for protein resolves

Constituents.	Patent flour.	Straight flour.	Low grade.	Dust room.	Ship stuff.	Bran.	Wheat.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Silica	2·33	1·28	0·50	1·34	0·49	0·97	1·04
Alumina	0·41	0·15	0·12	0·04	0·18	0·07	0·11
Ferric oxide	0·47	0·26	0·25	0·30	0·37	0·27	0·27
Potash	38·50	36·31	32·27	30·85	28·03	28·19	29·70
Soda	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Lime	5·59	5·65	4·51	3·53	2·80	2·50	3·10
Magnesia	4·39	6·44	9·33	12·90	13·27	14·76	13·23
Phosphoric acid	48·05	49·32	53·10	49·94	54·62	52·81	52·14
Sulphur trioxide	0·16	0·52	0·00	0·58	0·00	0·10	0·22
Chlorine	—	—	—	—	—	0·01	0·01
Zinc oxide	—	0·04	—	0·46	0·36	0·27	0·24
Total	99·90	99·97	100·08	99·94	100·12	99·95	100·06
Per cent. total ash in each	0·31	0·40	0·70	2·50	3·08	5·25	1·62

itself chiefly into the accurate determination of the nitrogen by the Kjeldahl method or one of its variations, and the multiplication of this by an accepted factor. For most feeding stuffs this factor is taken as 6·25, but for wheat and flour the usually accepted figure is 5·7. This factor of 5·7 has been criticised by Breese Jones (*Cer. Chem.*, 1926, **3**, 194), who points out that it is based on the nitrogen content of the two principal proteins in flour, gliadin and glutenin; this figure is accurate for the endosperm. If, however, the nitrogen content of the other bran proteins is taken into consideration, a figure of 6·31 is obtained for the bran, and 5·80 for the embryo. These figures give for the whole wheat a conversion factor of 5·83.

The errors that can creep into the accurate determination of the nitrogen content of a cereal are many. They have been emphasised by Coleman, Fellows and Dixon (*Cer. Chem.*, 1925, **2**, 132), Whitcomb and Lewis (*Cer. Chem.*, 1926, **3**, 232), and the Reports of the Committee on Methods of the American Association of Cereal Chemists (*Cer. Chem.*, 1925, **2**, 235; 1926, **3**, 254).

The necessity for carefully standardised solutions and measuring apparatus is obvious. For flour and most ground cereals (whole grains should be finely ground up), 1 grm. of material is sufficient. It is usual to employ the Kjeldahl-Gunning variation and to add to the substance about 8 grms. of nitrogen-free potassium or sodium sulphate. To these are added 20 c.c. of pure concentrated sulphuric acid. A crystal of copper sulphate or a bead of mercury can be used with advantage to hasten the decomposition of the organic matter. This mixture is digested in the usual long-necked Kjeldahl flask for at least thirty minutes after the solution has become colourless. In some American laboratories, where many hundreds of protein tests on wheat are done daily, speed of digestion is very important, and potassium perchlorate has been suggested to shorten the time. Harrel and Lanning (*Cer. Chem.*, 1929, **6**, 72) have emphasised the importance of the quantity of sodium sulphate used, and suggest that for a given heat source the time required for complete digestion can be varied by changing the ratio of the sodium sulphate to the acid. They suggest that

low protein results, when a rapid digestion was employed, are often due to insufficient sodium sulphate. When the digestion time is restricted to thirty-five minutes they suggest the use of as much as 14 grms. of sodium sulphate. Similar results were obtained by thirty-five minutes' digestion with 14 grms. of sodium sulphate as by one hundred and thirty minutes' digestion with the usual 7·5 grms. of sodium sulphate. Even when as much as 20 grms. of sodium sulphate were used there was no evidence of loss of ammonia during the digestion (1 grm. of flour and 20 c.c. of concentrated sulphuric acid used throughout these tests). It is, nevertheless, probably the safest practice to use only 8 grms. of potassium sulphate, and not to shorten the digestion time much under one and half hours.

The second part of the operation, the addition of caustic soda and the distillation of the ammonia into excess of a measured quantity of standard acid, can either be done in the same flask or after transferring the acid liquid to another distillation flask. The former procedure is the more popular in America, but care must be taken to see that the excess of strong caustic soda is poured down the side of the flask and forms a bottom layer, or else loss of ammonia will occur. Only after the flask is connected with the condenser, the end of which is dipping under the standard acid, must the flask be shaken and its contents mixed. It is often easier to transfer the digested liquid to a 800-c.c. round-bottomed flask, which is connected with the condenser and acid, and into which slight excess of the strong caustic soda can be added from a dropping funnel. In this case the rubber bung of the distillation flask has two holes, one carrying the dropping funnel and the other the trap leading to the condenser and acid flask. The time of distillation depends on the design and arrangement of the apparatus, but normally it should take from one to one and a quarter hours, about 150 c.c. of distillate being collected. Either N 20 or N 40 acid can be used. The most convenient indicators are methyl red, cochineal and sodium alizarin sulphonate. When copper is used as the catalyst in the digestion, it is convenient, as it turns dark blue and then brown when excess of caustic soda has been added to the distillation flask. When

metallic mercury is used (0·6 to 0·8 grm.) the following procedure is advisable :

Dilute the digested liquor, after cooling, to about 200 c.c. with cold water. If the dilution with water has warmed the solution, cool again and add 25 c.c. of a potassium (or sodium) sulphide (K_2S) solution containing 40 grms. per litre to precipitate the mercury. One or 2 grms. of metallic zinc may be added, if desired, to prevent bumping, and finally the excess (usually 80 to 100 c.c.) of strong sodium hydroxide solution.

The necessity for running fairly frequent blanks and making the necessary allowance is obvious, as all the reagents may contain traces of nitrogen. The findings of Coleman, Fellows and Dixon (*Cer. Chem.*, 1925, **2**, 132), after investigating protein tests done on standard samples in over forty-five mills, are of particular interest. Their conclusions were :

" Twenty c.c. of concentrated sulphuric acid are necessary to digest and retain all the ammonia liberated from a 1-grm. sample of wheat at the intensity of heat usually employed by most laboratories. Twenty-five c.c. are necessary when a 2-grm. sample is used.

" Heat is the most important factor in digesting wheat samples, as it controls the time a sample should be digested.

" Yellow and red oxides of mercury are equivalent in all quantities as catalytic agents, of which 0·5 grm. gives satisfactory results.

" Mercuric sulphate gives slightly lower results than the other two salts studied. Sodium sulphate and potassium sulphate are equal as catalytic agents. Ten grms. of either give best results when 20 c.c. of acid are used. Sodium sulphate, however, has the disadvantage of forming a salt cake with the usual amounts of sulphuric acid used for digestion. This salt cake can be prevented by using a mixture of 60 per cent. potassium sulphate and 40 per cent. sodium sulphate.

" Copper sulphate as a catalytic agent is of little value at low intensities of heat. A 2-grm. sample gives slightly higher results than a 1-grm. sample. From an error standpoint, the advantage of using a 2-grm. sample is not great enough to warrant its use.

" Considering the extra time needed to digest a 2-grm. sample, the 2-grm. sample is undesirable.

" The Kjeldahl method is the only method with which wheat samples can be completely oxidised in less than an hour at all intensities of heat. The Gunning method and the Kansas City Protein Referee Board Methods are not satisfactory at low heats.

" No difference in results can be attributed to methods if the heat is sufficiently intense. Wheat can be completely oxidised in forty-five

minutes at high heat, and in sixty minutes at medium heat, when a 1-grm. sample is used. It takes 30 per cent. more time to digest a 2-grm. sample.

"The heat intensities used were as follows:

"Low heat—50 c.c. water evaporated from 200 c.c. in twenty minutes.

"Medium heat—100 c.c. water evaporated from 200 c.c. in twenty minutes.

"High heat—150 c.c. water evaporated from 200 c.c. in twenty minutes.

"The time was recorded when the water began to boil.

"Not less than 30 grms. of wheat should be ground for protein tests to secure results representative of the bulk sample. Errors of as much as 0·3 per cent. will result if smaller samples are used.

"Wheat should be ground whenever possible. Results from whole seed are irregular when compared with data obtained from carefully ground seed.

"Great care should be taken to retain the moisture in the sample, as changes in moisture induce changes in the protein test results. Differences of 0·6 per cent. have been noted in these investigations.

"In the distillation process careful attention should be given to the use of traps and the making of blank determinations.

"At least 100 c.c. of distillate should be collected in acid which will hold the equivalent of 35 to 70 mg. of nitrogen, depending upon whether a 1- or 2-grm. sample is used. There is no choice between sulphuric and hydrochloric as receiving acids.

"Boric acid will lose ammonia at 40° C., the loss increasing with the rise in temperature. Boric acid solution containing ammonia must be titrated within four hours in order to incur no losses of ammonia. Sodium thiosulphate is an acceptable substitute for sodium or potassium sulphide. Careful attention should also be given to standardising normal solutions. The error in making such standards is much too large. Errors in terms of protein equal 0·35 per cent.

"The use of potassium acid phthalate as a standard solution is recommended."

Protein Distribution in Wheaten Flour.—There have been many methods suggested for the separation of the proteins of flour into their different components, such as gliadin and glutenin. Normally, three separations are made—proteins soluble in 5 per cent. potassium sulphate solution, proteins soluble in 70 per cent. alcohol, generally assumed to be gliadin, and the difference between the total protein and the sum of the above two, assumed to be glutenin.

There is some doubt whether gliadin and glutenin are very clear chemical entities. Up to the last eight years, the 5 per cent. potassium sulphate solution extraction and the 70 per cent. alcoholic extraction were made on separate samples of the flour. Sharp and Gortner (*Minn. Agr. Exp. Sta. Tech. Bull.*, 1923, **19**) pointed out that these two solvents partly extract the same material, and, as the glutenin value is obtained by difference from the total protein, it is thereby reported too low. They, therefore, recommend that the glutenin content would be most accurately determined by subtracting from the total protein content of the flour the sum of the amounts of protein extracted by 5 per cent. potassium sulphate followed by 70 per cent. alcohol (on the same sample). The temperature and time of the extraction, however, influence all such results. At the best, therefore, all such work is only approximate. Gortner, Hoffmann and Sinclair (*Cer. Chem.*, 1929, **6**, 1) have investigated the peptising action of nineteen various inorganic salt solutions (such as NaCl, KCl, KBr, KI, $MgCl_2$, $CaCl_2$, etc.). They have shown that there is a huge difference in the amount of protein extracted by these different salts, and this also depends on the concentration of the salt solution used.

It is obvious, therefore, that any division of the flour proteins obtained in this way is arbitrary. There seems some evidence to suggest that of all the flour proteins, glutenin is the most distinctive. It appears to be more of a chemical compound with distinctive characteristics than merely something extractable by a certain solvent under certain conditions. The latest work by Blish and Sandstedt (*Cer. Chem.*, 1926, **6**, 494) suggests, however, that glutenin is a derived rather than a naturally occurring protein. Definite procedures have been suggested for its determination. In view of this and the ease with which the extraction with 5 per cent. potassium sulphate solution can be standardised, the following procedure is likely to give the best information and most accurate results:

Proteins Soluble in 5 per cent. Potassium Sulphate. Six grms. of flour are taken and moderately shaken in a mechanical mixer at 20° C. ± 2° with 100 c.c. of 5 per cent. potassium sulphate solution (nitrogen

free) for exactly one hour. The solution is allowed to settle for about thirty minutes, and is then centrifuged, and finally filtered through a No. 5 Whatman paper. The centrifuging is done in such a way that the undissolved matter does not remove, by ultra-filtration, any of the dissolved protein. Fifty c.c. of the filtrate (equivalent to 3 grms. of flour) are taken for a nitrogen estimation. To the 50 c.c. in a long Kjeldahl flask are added a few drops of strong sulphuric acid, and the whole is then evaporated (in the Kjeldahl flask) to a few c.c. To this are added the usual quantities of sulphuric acid, potassium sulphate and a crystal of copper sulphate, and the digestion and subsequent distillation carried out in the usual manner. The protein is returned as the nitrogen percentage, multiplied by 5·7.

Glutenin. This is determined by one of the methods given below.

Gliadin. What is termed "gliadin" may then be best determined by the difference between the total protein and the sum of the glutenin and proteins soluble in potassium sulphate. The result thus obtained, however, only agrees roughly with the direct determination of gliadin by extraction with 70 per cent. alcohol (*Association Official Agricultural Chemists' Method*), if the successive solution procedure suggested by Sharp and Gortner is employed.

Glutenin. A direct determination of glutenin was suggested by Blish and Sandstedt (*Cer. Chem.*, 1925, **2**, 57):

"Eight grms. of flour are weighed into a dry 200-c.c. Kohlrausch flask, or any 200-c.c. flask with extra capacity. The flour is thoroughly mixed with 50 c.c. of water, after which 5 c.c. of normal NaOH are added from a pipette, during which process the flask and contents are vigorously rotated. The mixture is then shaken, and the shaking is repeated at ten-minute intervals for one hour (less time would probably suffice). Pure, acetone-free methyl alcohol (96 to 99 per cent.) is now added in portions of about 50 c.c. at a time, with shaking after each addition, until the 200 mark is reached. One should then add 5 c.c. of methyl alcohol in excess, which compensates closely enough for the volume occupied by the flour. The starch then settles rapidly to the bottom of the flask, leaving a fairly clear supernatant liquid which holds in solution all of the flour protein. This solution contains approximately 70 per cent. methyl alcohol by volume, and is less than 0·025 normal with respect to NaOH. It may be rapidly decanted through a cotton plug. A 50-c.c. portion of this liquid (equivalent to 2 grms. of flour) is pipetted off into a 100-c.c. Erlenmeyer flask, and a few drops of bromthymol blue are added. The glutenin is then precipitated by adding 0·2*N* HCl from a burette, with constant shaking, until a distinct colour change occurs. Then a drop or two more acid should be added, until a light olive colour is reached, corresponding to a *p*H of about 6·4. However, the precipitation seems to be complete and quantitative over

a considerable range, between *p*H = 6·0 and 6·8, as indicated by bromthymol blue under existing conditions. After a few minutes the glutenin will settle, leaving a clear supernatant liquid. At the end of an hour or two, the contents of the flask are poured into a 100-c.c. centrifuge tube and whirled in the centrifuge for ten minutes. The clear liquid is then completely poured off, and the compact disc of glutenin detaches itself in one piece from the bottom of the centrifuge tube when a little distilled water is added. This is poured into a Kjeldahl flask. The nitrogen is determined in the usual manner, and multiplied by 5·7 for conversion to glutenin. It was found that when the glutenin precipitate is first poured into the centrifuge tube, a very slight portion of the finely divided solid material adheres to the sides of the tube above the liquid, and remains dry upon the upper-side portion of the tube after centrifuging. A very slight amount of the glutenin may also remain in the Erlenmeyer flask from which the mixture is poured. However, the glutenin nitrogen thus lost is, for all practical purposes, compensated for by the nitrogen in the small amount of filtrate which wets the glutenin after the centrifuge has thrown it down, and which cannot be washed out. Added to this is the small portion of filtrate which wets the inside of the centrifuge tube after the bulk of the filtrate has been poured off. The slight errors thus compensating each other, the only correction necessary is the one which is always occasioned by small amounts of nitrogen which may be found in the usual Kjeldahl reagents as determined by the customary 'blank' determinations."

An alternative method to this was suggested by Blish, Abbot and Platenius (*Cer. Chem.*, 1927, **4**, 129). In essence this is as follows :

Eight grms. of flour are placed in a 200-c.c. graduated flask and 0·2 grm. solid barium hydroxide is added. To this are added 50 c.c. of distilled water and thorough mixing effected. The whole is allowed to stand for exactly one hour, shaking at ten-minute intervals. Finally it is made up to the mark, with 5 c.c. extra (to compensate for the flour), with pure methyl alcohol. It is then mixed vigorously. After standing to allow starch to settle, it is filtered quickly through a cotton plug. An aliquot portion of 50 c.c. is transferred to a Kjeldahl flask for the ordinary nitrogen determination. It is important that the time between the adding of the methyl alcohol and the taking of the aliquot portion should not be more than fifteen minutes, as otherwise gliadin may be precipitated. The glutenin is given by the difference between the total protein and that found in the solution taken. This result agrees closely with that of the original Blish and Sandstedt method, which must be followed very closely if consistent results are to be obtained. The

barium hydroxide method is said to be superior to the original Blish and Sandstedt method from the standpoints of simplicity, economy of time as well as equipment.

Amino Nitrogen. It is often useful to be able to estimate the nitrogen present in a cereal in the form of free amino acid. Numerous methods have been proposed for this, many depending on the precipitation of the proteins from solution, thus leaving the non-protein substances. Both copper hydroxide (Blish, *J. Biol. Chem.*, **33,** 551) and stannous chloride have been suggested as precipitating agents. Stannous chloride was first suggested by Scherning (*Z. anal. Chem.*, 1897, **36,** 643), and later used, amongst others, by Sorensen (*Biochem. Z.*, 1909, **21**, 131, *cf.* p. 292) and Olsen (*Thesis on Proteases of Bread Yeast*, University of Minnesota).

In using stannous chloride, 50 grms. of tin are dissolved in fairly concentrated hydrochloric acid which contains a few drops of platinic chloride to hasten the reaction. Six c.c. of a solution obtained by evaporating this to dryness and then making up to 500 c.c. are used to precipitate the proteins. A convenient suspension of flour or dough of known weight is taken and diluted to 500 c.c. To 50 c.c. of this are added 6 c.c. of the stannous chloride solution. Precipitation is said to be best at about *p*H 5·9. Caustic soda may be conveniently used to reach this point, the indicator brom-cresol purple turning from yellow to faint blue-purple. An aliquot portion of the centrifuged liquid can then be submitted to the usual Kjeldahl determination. This method and the copper hydroxide method are described by Bailey and Johnson (*Cer. Chem.*, 1924, **1**, 376). Similarly, sodium tungstate may be used to precipitate the proteins as in the maltose estimation (see p. 228). Swanson and Tague (*J. Amer. Chem. Soc.*, 1916, **38,** 1 ; 1917, **39,** 482) have adopted the Sorensen formol titration for flour work ; this is carried out thus :

Filter as well as possible 50 to 70 c.c. of the flour extract (No. 5 Whatman paper is convenient). Two portions, each of 20 c.c., are taken. To the one, 2 c.c. of a stock formaldehyde solution (approximately 40 per cent.) and 1 c.c. of a solution made by dissolving 0·05 grm. of thymolphthalein in 100 c.c. of 97 per cent. neutral alcohol are added.

After standing fifteen minutes, this is titrated against *N* 20 barium hydroxide. The other portion of 20 c.c. is titrated against *N* 20 barium hydroxide direct, 1 c.c. of a 0·5 per cent. neutral alcoholic solution of phenolphthalein being used as indicator. After allowing for the acidity of the formaldehyde (previously determined), the difference in the two titrations is due to the amino acids present. The phenolphthalein solution should be titrated to a faint pink, and the thymolphthalein to the first tinge of blue. If *N* 20 alkali be employed, the difference, multiplied by 0·7, gives the milligrams of titratable nitrogen in the solution. The amino acids under these conditions combine with the aldehyde, and, as the basicity of the $-NH_2$ group is destroyed, the —COOH group can be titrated directly.

The above methods can naturally be applied to cereals other than wheaten flour.

Gluten.—The determination of gluten in flour is one of the oldest of all tests. Its inaccuracies have been referred to previously, as well as the attempts to use standard wash-water. Gluten tests are only strictly comparable when made by the same operator, using the same conditions. The errors inherent in the method, which at the best is an approximate and crude one, led to its condemnation by Jessen-Hansen (*C. R. Lab. Carlsberg*, 1911, **10**, 17) and others. Dill and Alsberg (*Cer. Chem.*, 1924, **1**, 222) suggested that the use of a definite wash-water containing 0·1 per cent. of mixed phosphates, at a *p*H of 6·8, should be adopted, in order to standardise the gluten-washing test and to remove anomalies due to variations in the composition of tap-water.

Kent-Jones and Herd (*Analyst*, 1927, **52**, 439) collected gluten results on flour samples sent to eight chemists, each operator using ordinary tap-water and the special solution of Dill and Alsberg. They found that the use of this standard wash-water did not eliminate the differences between the results obtained by different observers on the same flour. It was shown that there is a strong personal factor in this test, and that this is the main cause of the variations obtained; further, it was demonstrated that each individual observer gets essentially consistent results, in that the ratio between the nitrogen of the flour and the dried gluten content is approximately constant for each operator.

Any method can be adopted for the gluten determination so long as it is always adhered to. A generally accepted procedure is as follows, although other methods are employed :

Twenty grms. of flour are taken and made up into a dough with the requisite amount of water. This can either be done in a special small Pfleiderer machine or by hand (or, rather, by spatula). The latter method is quick and accurate, after a little practice. The dough is then placed in a small bowl of ordinary tap-water at about 60° to 65° F., and allowed to stand for one hour. The water tap is then turned on, so that it is at a certain standard constant flow (about 250 c.c. per minute). The dough is then kneaded by hand under this flowing water. The dough will tend to break up at first, and some care and skill are required, which come with practice. The dough should not be enclosed in a covering of muslin or suchlike material, as in such cases the test is deceptive and unreliable. After a time most of the starch has been washed away as a milky suspension. The remaining piece of gluten becomes more and more cohesive and coherent. While the washing away of the starch proceeds, little pieces of gluten tend to break off (especially at the commencement), and to prevent loss of these it is best to have immediately beneath the dough a stretched-out piece of fine silk. This lets through into the waste sink the milky suspension of starch, but catches the pieces of gluten, which can be picked up at a later stage and joined on to the main gluten residue. The washing should occupy from start to finish exactly ten minutes.

At the end of this ten minutes there only remains a more or less elastic piece of gluten. This is placed on a previously dried and weighed piece of filter-paper and put in the water oven at 99° C. for exactly twenty-four hours. Then it is weighed, and the weight of the dried gluten and paper, minus the weight of the dried paper originally obtained, multiplied by 5, gives the percentage of dried gluten.

Even more important than the actual amount of dried gluten is the quality of the gluten. Many glutens of poor quality will, during the drying, run out quite flat. Good glutens will stand up.

Sugar.—The determination of sugar as maltose in wheaten flour has attracted considerable attention of late years. The recent methods devised undoubtedly do give useful information as to the possible behaviour of flour in fermentation and baking. Flour contains many kinds of sugars—maltose, cane-sugar, dextrose, etc. If a flour extract be boiled with Fehling's solution, a certain amount of copper oxide will be deposited. As, however, cane-sugar is also present, an additional quantity of copper will be

reduced if there is a preliminary boiling with dilute acid. One of the main difficulties in dealing with sugar determinations in any cereal extract is due to the fact that the diastatic enzymes present are acting all the time and are particularly active in the presence of excess of water. It is, therefore, difficult to state exactly how much sugar there is existent in any flour, as, during the extraction period, this amount is constantly increasing. Temperature of the extraction also affects the activity of the sugar-producing enzymes. It is, therefore, essential to have a uniform temperature, time and method of extraction.

As has been seen, the diastatic power of a flour determines one of the two main factors as to its suitability for bread-making purposes. At one time attempts were made to determine the diastatic power by the usual Lintner method, *i.e.*, by allowing extracts to act on definite quantities of soluble starch. It was found, however, that the results were of little guidance as to whether the flour, when doughed in the ordinary way with water, yeast and salt, would gas sufficiently throughout the fermentation period. Rumsey (*Amer. Inst. Baking Bull.*, 8), however, introduced a useful autolytic method. The particular value of this was that it could be correlated fairly well with the behaviour of the dough in fermentation. This has been commented upon by Kent-Jones (*Modern Cereal Chemistry*, p. 368) and Ritter (*Z. für das gesamte Getreidewesen*, 1928, 13). The method proposed by Rumsey is as follows:

"Ten grms. of flour are weighed out and transferred to a 250 to 300 c.c. Erlenmeyer flask. This is placed in a water thermostat and brought to a temperature of exactly 27° C. A flask containing a sufficient volume of distilled water is also placed in the bath and kept at 27° C. for subsequent use. By means of a pipette, 100 c.c. of the distilled water are run into the sample, while the flask is rotated to obtain a thorough suspension of the flour. The last few c.c. in the pipette are allowed to rinse down the material from the sides of the flask. The flask is quickly replaced in the thermostat, stoppered loosely, and allowed to remain exactly sixty minutes. A few minutes after starting the digestion, the flask is rotated to stir up the suspension and hasten the equalisation of temperatures, and this shaking is repeated every quarter of an hour. At the end of the digestion period the contents of the flask are quickly rinsed into a 200-c.c. graduated flask, diluted

to about 175 c.c., and clarified. To clarify, first make sure that the solution is neutral or slightly alkaline. Five drops of 0·04 per cent. thymol blue serve as a convenient indicator, appearing cream yellow in colour when neutral, and blue when slightly alkaline. Add 3 c.c. of a 15 per cent. solution of sodium tungstate ($Na_2WO_4 2H_2O$) and mix thoroughly with the flour suspension. Then add, drop by drop, from a 1-ml. graduated pipette with constant shaking, sufficient concentrated sulphuric acid to turn the indicator a decided pink colour, with 2 to 3 drops in excess. Four-tenths of a ml. (0·4 c.c.) are usually sufficient if the original flour suspension was nearly neutral. This clarification likewise serves to stop the enzymic activity and prevents further change in sugar content. Dilute to the mark (200 c.c.), shake thoroughly, pour into centrifugal cups, and whirl for about five minutes. By means of a calibrated pipette, transfer 50 c.c. of the clear supernatant liquid to a 400-c.c. Pyrex beaker for the determination of the reducing sugars by the Munson-Walker official method. A blank is also run at the same time on the sample to correct for the natural reducing sugars in the flour. It likewise gives a measure of these reducing sugars. To prepare the blank, mix 100 c.c. of water at 27° C. and 10 grms. of flour, and immediately inhibit diastatic activity by clarifying with the sodium tungstate in the manner just described. The blank determination is then carried out in the same manner as the samples, except that the addition of the 0·4 c.c. of concentrated sulphuric acid is omitted on dilution to volume. It does no harm to allow the clarified solution in the beakers to stand an hour or two until other samples are ready. When making the Fehling reduction, the excess acidity of the solution can conveniently be neutralised by using a predetermined number of drops of strong caustic soda. The cuprous oxide Cu_2O is filtered, washed, dried and weighed in a Gooch crucible."

In ordinary practice both Kent-Jones and Ritter suggest that there is little need for the determination and deduction of pre-existing sugar. Kent-Jones, therefore, determines the maltose present at the end of the hour's incubation at 27° C. and applies the Lane and Eynon methylene blue volumetric method (*J. Soc. Chem. Ind.*, 1923, **42**, 32T) for the actual Fehling's estimation. Ritter prefers the procedure of Bertrand, which depends on the principle that the copper oxide can be dissolved by means of a sulphuric acid ferric sulphate solution, and the ferrosulphate thus formed can be titrated against *N*/10 potassium permanganate —

$$Cu_2O + Fe_2(SO_4)_3 + H_2SO_4 = H_2O + 2CuSO_4 + 2FeSO_4$$
$$10FeSO_4 + 2KMnO_4 + 8H_2SO_4 = 8H_2O + 5Fe_2(SO_4)_3 + 2MnSO_4 + K_2SO_4$$

The extract and the Fehling's solution are boiled for exactly four minutes, and the precipitated copper oxide is filtered off (with the aid of the pump) on a Gooch crucible and washed. The greater part of the washing can best be done by decantation in the flask. The flask is then ready for the dissolving of the copper oxide, which is done by adding 20 c.c. of a solution made by dissolving 50 grms. of ferric sulphate in water, adding 200 c.c. of concentrated sulphuric acid, and making up to the litre with distilled water. These 20 c.c. are slowly passed through the Gooch crucible, whereby all the copper oxide is dissolved. If any doubt remains, a little more of the warmed acid ferric sulphate solution may be poured through the Gooch crucible. The crucible is then well washed with distilled water. The runnings, now in the flask, are titrated against *N* 10 potassium permanganate to a faint but definite pink. One c.c. of *N* 10 $KMnO_4$ corresponds to 0·006357 grm. of copper. The end-point is quite sharp, and there is much to commend the method. The usual tables can then be employed to calculate the sugar content.

The figure obtained, whatever analytical procedure is employed, is the percentage of reducing sugar originally in the flour, plus that made in the one hour at 27° C. by the diastase of the flour. It has been found that the gassing power of the flour and the power of the loaf to take the fire of the oven (*i.e.*, to colour on the crust) are, in general, related to this figure. If over 1·5 per cent., the flour should be satisfactory in these respects. If below 1·5 per cent., there may be complaints of the loaves not taking the fire, etc. This latter point is more important in the south of England than in the north.

Just as it is dangerous for the maltose figure to be much below 1·5 per cent., so it is dangerous if the figure becomes too high. In this case the dough may have the feeling of one made from sprouted wheat and is inclined to be sticky. Although it works freely and produces gas rapidly, the loaves tend to collapse as they are taken from the oven, and the crumb may feel sticky. It is advisable, therefore, to keep the maltose content under 2·3 per cent., as, otherwise, some of the characteristics mentioned above may be noticed, particularly if unfavourable conditions

prevail during baking. In this connection, trouble is sometimes experienced when a large proportion of low-grade Manitoba or sprouted English wheat is incorporated in the blend. A miller's blend should be made with as much regard for maltose content as for gluten content; there is some evidence to suggest that high maltose figures are not so dangerous in some seasons as in others.

The usual remedy for insufficient maltose is to have in the blend enough of the wheats which gas well, such as Durum, which often has a maltose figure of over 2 per cent. Some wheats yield flour of low maltose figure, and hence of very poor gassing power. For instance, Australian and Plate wheat often have maltose figures below 1 per cent. In such cases the blend should not contain much of these wheats, unless the remainder of the blend has a high maltose figure, so that it averages out between 1·5 and 2·3 per cent. High gluten content seems to counteract many of the disadvantages of low maltose figures.

Fat.—Fat is usually estimated by an ether, petroleum spirit or mixed ether extraction, but there is some doubt as to whether a direct extraction should be made or the material submitted in the first place to an acid or alkaline hydrolysis. In feeding stuffs, the official English method is to extract with petroleum spirit (b.p. 40 to 60 C.) in a Soxhlet apparatus for three hours. The residue is then removed, ground up with fine sand and extracted with the same solvent for another hour. The petroleum spirit is removed and the fat weighed, after drying to constant weight in a water oven.

The A.O.A.C. advises for feeding stuffs the direct extraction of the dried sample with anhydrous ether for sixteen hours. The extract is then evaporated and dried to constant weight. Until recent years, the fats in flour and suchlike cereal products have been directly extracted with ether. Nowadays, for flour and baked goods, the acid hydrolysis method is recommended. At one time alkaline hydrolysis was preferred for flour, but the simple, straightforward and quick acid method is now in general use for most cereal products. This is carried out as follows:

Two grms. of the ground cereal are placed in a 50-c.c. beaker, and 2 c.c. of 95 per cent. alcohol added. This is mixed so that all particles

are thoroughly wetted. Ten c.c. of HCl (25 to 11) are added and complete mixing effected. The beaker is then warmed to 70° to 80° C. in a water-bath for thirty to forty minutes, or sufficient time to destroy starch and other material. Ten c.c. of 95 per cent. alcohol are added and the whole is cooled. It is then transferred to a stoppered cylinder, the beaker being well rinsed out with a total 25 c.c. of washed ethyl ether. The whole is mixed in the cylinder, 25 c.c. of petroleum spirit added and then re-mixed thoroughly. It is allowed to stand till the top ethereal layer clears. This is removed by filtering through a cotton plug into a tared beaker. The extraction is repeated twice more, 15 c.c. of washed ethyl ether being used each time and 15 c.c. petroleum spirit added separately, with mixing after each addition.

These solvents, after clearing, are similarly run off through the cotton plug. The total filtered extract is evaporated to dryness and weighed, after drying to constant weight, in a water oven. It is recommended that all weighings done in this method should be carried out after the beaker has obtained equilibrium with the air by standing in the balance case (desiccator not used). The acid hydrolysis method gives appreciably higher results than the direct extraction method.

Possibly the extract may contain some lipoid fat, but only after hydrolysis which has freed it from the choline base. Fats obtained in this way are essentially free from nitrogen and phosphorus.

Lipoids are fatty substances containing nitrogen or nitrogen and phosphorus. Phosphatides are lipoids containing phosphorus only. These are usually estimated by the official method of the A.O.A.C. This, in essence, is as follows :

Five grms. of material are placed in a wide-mouthed glass-stoppered bottle with 15 c.c. of 70 per cent. alcohol. This is placed in a water bath at 70° to 80° C. for fifteen minutes. After removing, 27 c.c. of 95 per cent. alcohol are added and vigorously shaken for two minutes. After cooling, 45 c.c. of ether are added, and the shaking continued for a further five minutes. The liquid layer is removed by centrifuging, so as to give a clear layer over the solid, but the latter should not be packed too tightly. Three further extractions are carried out, each with 20 c.c. of ether and similarly removed by centrifuging. The total extracts are evaporated to dryness in a steam bath, and placed in a water oven for five minutes to remove traces of water. To this are added a few c.c. of chloroform which dissolves the fatty material. This is then filtered off through a cotton plug into a tared bowl, the chloroform extraction and filtration being repeated at least twice. The extract is then evaporated to dryness and finally weighed after drying to constant weight in a water oven.

Starch. The determination of starch in cereal products presents many difficulties, and accurate determinations cannot be made. The difficulty is enhanced by the fact that products intermediate between sugars, cellulose and starch exist, and the various analytical procedures include different amounts of these substances. In general, there are three methods employed :

(*a*) In flour, the direct washing out of the starch.

(*b*) The conversion of starch by hydrolysis (acids or enzymes) into sugar, which can then be determined.

(*c*) The dissolving out and re-precipitation of the starch, as, for instance, in Rask's method described later.

The direct washing out of starch is, of course, only approximate, but if a dough is made from 1 grm. of flour and this is washed out, after standing, in a muslin bag immersed in a beaker of water, practically all the starch can be transferred to the water. A second and third washing in fresh water approximately completes the operation. The cloudy waters are then collected and mixed, and the starch allowed to subside for several hours. The supernatant liquid can then be decanted, and the starch rewashed by decantation. The starch is finally transferred to a No. 5 Whatman filter paper, washed with alcohol and ether, allowed to dry at 40° C., and finally in a water oven at 98° C. to constant weight, the filter paper being enclosed in a weighing bottle. Duplicate results on flour should come, with practice, within 1 per cent., but the method is obviously open to much criticism from an analytical point of view.

There are many hydrolysis and conversion methods. There is, for instance, the method of the A.O.A.C., in which conversion into sugar is carried out under exact conditions, using both diastase and then hydrochloric acid. Such a method for flour is described in *Modern Cereal Chemistry*, pp. 335–336. Among the many proposals of this nature, the Ling, Nanji and Harper method (*J. Inst. Brew.*, **30**, 838) is perhaps the most encouraging. In essence, this is as follows :

The sample (for a crushed grain 5 grms. are advised, but for flour 2 grms. are ample) is extracted in a Soxhlet extractor with 50 per cent. alcohol, and the residue gelatinised with hot water in the usual way. The

paste is cooled to 50° C., and a quantity of freshly precipitated barley diastase added. The mixture is then kept at 50° C. for twelve hours, boiled, cooled and made up to 500 c.c. The sugar content of an aliquot portion is then determined by means of Fehling's solution or iodine. The barley diastase is obtained by extracting 100 grms. of finely ground barley with 250 c.c. of water, filtering and precipitating the diastase in 10 grms. of extract with twice the volume of alcohol. The wet precipitate is suspended in water and is ready for the test, as indicated above. Simultaneously with this procedure, a blank experiment with a similar amount of the barley diastase is made on 2 grms. of pure potato starch of known moisture content. Since potato starch contains only amylose and amylopectin, the percentage of starch in the sample can be calculated by the following formula :

$$\frac{100\,M}{M^1}$$

where M represents the maltose in the sample expressed on 100 parts of dry material and M^1 the maltose produced from a similar amount of potato starch (dry basis).

The Rask method is not as easy to manipulate as it appears. Care must be taken to ensure that lumps are not formed whereby starch may escape solution, resulting in low determinations ; filtering difficulties may also occur. Some difficulty is experienced in obtaining satisfactory duplicates. The flour or starch-containing material is extracted with ether, 10 per cent. alcohol and water successively : the starch is then dispersed in hydrochloric acid solution (20·5 to 21 grms. of HCl per 100 c.c. solution), made up to a known volume, and filtered through asbestos. To a given aliquot portion of the filtrate is added 96 per cent. alcohol, which precipitates the starch : this precipitate is allowed to settle, and the supernatant liquid decanted through a tared Gooch crucible ; the starch is washed by decantation with 70 per cent. alcohol (three times) and finally with 96 per cent. alcohol, until all the hydrochloric acid is removed. The precipitated starch is then transferred to the crucible by means of 96 per cent. alcohol, washed with anhydrous ether and dried to constant weight. The starch must not be left in contact with the hydrochloric acid for more than thirty-five minutes, on account of possible hydrolysis.

Fibre. — Fibre determinations depend entirely upon the empirical conditions under which they are made, and hence official methods

are mainly employed. It is usual to boil the sample with 1·25 per cent. acid, and then with the same strength alkali, definite conditions being observed. These may be best obtained direct from the various communications (*e.g.*, *Association of Official Agricultural Chemists' Methods of Analysis*, 1925).

The official English method (Ministry of Agriculture and Fisheries, Fertilisers and Feeding Stuffs Regulations, 1928) is as follows :

"Two or 3 grms., accurately weighed, shall be extracted with petroleum spirit (b.p. 40 to 60 C.) in an extraction apparatus, or at least three times by stirring, settling and decantation, and the dry residue transferred to a conical 1,000-ml. flask. The material must not be further ground during extraction. A volume of 200 ml. of a solution containing 1·25 grms. of sulphuric acid (H_2SO_4) per 100 ml., measured at ordinary temperature and brought to boiling point, shall be added to the flask and heated. The contents of the flask must come to boiling within one minute and the boiling throughout must be gentle and continuous for exactly thirty minutes, the original volume being maintained. The flask shall be rotated every few minutes in order to mix the contents and remove particles from the sides. At the end of thirty minutes the flask shall be removed and the contents poured at once into the shallow layer of hot water remaining in a funnel fitted with a pump-plate, or alternatively into the similar layer remaining in a Buchner funnel. The funnel shall be prepared by cutting a piece of cotton cloth or filter-paper to cover the holes, so as to serve as a support for a disc of ordinary filter paper ; boiling water shall be poured into the funnel and allowed to remain until the funnel is hot, whereupon suction is applied. The experiment shall be discarded if the time of filtration of the bulk of the 200 ml. exceeds ten minutes. The residue shall be washed with boiling water until the washings are free from acid. The residue shall then be washed from the filter paper back into the flask with a volume of 200 ml. of a solution of sodium hydroxide, containing 1·25 grms. of sodium hydroxide (NaOH) per 100 ml. free or nearly free from sodium carbonate, measured at ordinary temperature and brought to boiling point. The contents of the flask shall be boiled for exactly thirty minutes, the precautions given for the treatment with acid being observed. At the end of thirty minutes the flask shall be removed and its contents immediately filtered through an ordinary filter paper. The residue collected on the filter paper shall be washed with boiling water, then with a solution of 1 per cent. hydrochloric acid and again with boiling water until free from acid. The residue shall then be washed twice with 95 per cent. alcohol, and three times

with ether. The residue shall then be transferred to a dried, weighed, ashless filter paper, dried at about 100° C. in an oven and weighed in its weighing bottle until constant in weight. The ash of the paper and contents shall be determined by incineration at a dull red heat. The weight of ash shall be subtracted from the increase of weight found on the paper and the difference shall be reported as fibre."

It is often more convenient and accurate, especially where the quantities are small, *e.g.*, in flour, to transfer the final residue (before washing with alcohol and ether) by means of a fine jet from a wash bottle to a weighed platinum bowl. By this means the alcohol and ether washings are dispensed with, as the whole can be fairly quickly evaporated to dryness on a water bath and finally dried to constant weight in a water oven. The ash can then be determined directly by ignition of the bowl, and allowed for.

Hydrogen Ion Concentration.— Although by no means a routine test, the determination of the hydrogen ion concentration of a cereal is occasionally required. In such cases either a suspension of the product (ground if necessary) in pure distilled water is used or a definite extract is made. Either the colorimetric procedure can then be followed or an electrometric determination made.

There is some difference of opinion as to whether a suspension or a filtered extract should be used, and, further, what concentration should be employed. The concentration makes but little difference to the actual hydrogen ion concentration, but does affect the buffer value, as judged by the change in hydrogen ion concentration on the addition of a definite quantity of acid. Bailey and Peterson (*J. Ind. Eng. Chem.*, 1921, **13**, 916) suggested that filtered extracts should be used, and that the original *p*H is scarcely affected by either time of extraction or temperature. Kent-Jones (*Modern Cereal Chemistry*, p. 116) states that there is no practical difference between the original *p*H of the suspension and that of the filtered extract, although differences arise on the addition of acids, etc. General methods of the determination of hydrogen ion concentration in flour are discussed and described by Kent-Jones (*Modern Cereal Chemistry*, pp. 369–377).

If the colorimetric method is employed, standard buffered solutions of known *p*H are compared with the unknown flour

extract, similar amounts of indicator (normally methyl red) being used, and the colours matched. For this purpose a Walpole comparator is useful. The standard *p*H solutions are usually obtained from known mixtures of potassium acid phthalate and sodium hydroxide.

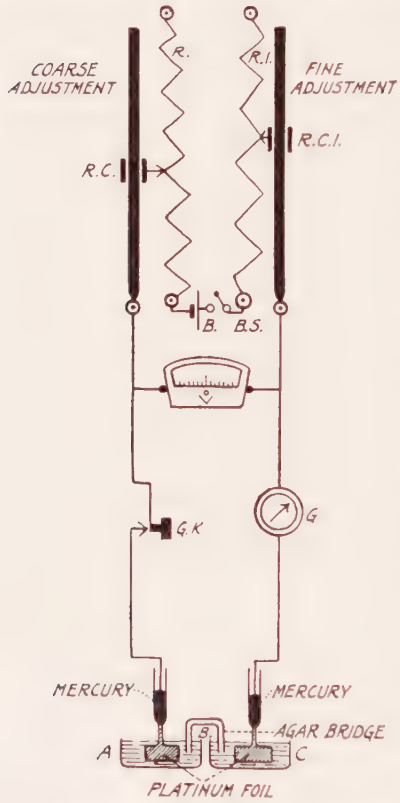

FIG. 9. Apparatus for determining the hydrogen ion concentration of cereals.

When the more exact method is employed, viz., when the electric potential is measured, either a hydrogen electrode is used or the quinhydrone method employed. Bailey (*Amer. Chem. Soc.*, 1920, **42**, 54) has devised a special form of hydrogen electrode suitable for flour and other plant extracts, but Halton and Fisher (*Cer. Chem.*, 1928, **5**, 445) prefer to use a modified Bunker type of electrode. These investigators suggest that concordant results are obtained with centrifuged extracts and decantates from suspensions, but that filtered extracts give slightly low values, and suspensions always high values. Denham and Scott Blair (*Cer. Chem.*, 1926, **3**, 158) applied the quinhydrone method to flour work. As they find a slight but continuous potential drift in using suspensions, owing to the gradual leaching out of substances, standard extraction methods (in opposition to Halton and Fisher) are advocated. Denham and Scott Blair, as well as Kent-Jones, normally employ platinum electrodes, but Halton and Fisher prefer gold ones. Finality, therefore, has not been reached.

The quinhydrone method is, however, simple to manipulate, and is rapidly displacing the older methods employing hydrogen. A simple electrical equipment (Kent-Jones, *Modern Cereal Chemistry*, pp. 374–376) consists of an accumulator (a 2-volt Exide one is convenient), marked B in Fig. 9, a switch, marked B.S., two variable resistances (one for coarse adjustment and one for fine), a galvanometer G (of high resistance and capable of detecting minute currents), and a direct-reading large-scaled Weston millivoltmeter V. Such sets can be easily put together or purchased from most scientific instrument dealers. The electrodes immersed in the solutions held in A and C are of bright platinum or gold. As they require constant re-annealing, and as thin gold tends to melt fairly easily, it is usually more convenient to use platinum. The electrodes can be about as small as $\frac{1}{4}$ inch square, and are sealed into test-tubes, so that connection can be easily made by pouring mercury into these. A contains the flour extract, the *p*H of which it is desired to find, and C a buffer solution of known *p*H value. A solution *p*H 3·60 is convenient, and is made by adding 50 c.c. of *M* 5 acid potassium phthalate solution to 6 c.c. of *M* 5 HCl and diluting with pure distilled water to 200 c.c. The electrode in A is connected with the key, and the other electrode with the galvanometer (see Fig. 9). No hydrogen, calomel cell, etc., are required. Just before the determination is made, a little quinhydrone is placed in each of the solutions in A and C (just enough to go on the end of a penknife or spatula). This should be stirred for a second or two and allowed to rest about a minute or so before the electrical measurement is taken. Equilibrium is reached, as a rule, quite quickly (one to two minutes). It should be remembered that a little time is wanted, as some of the quinhydrone must dissolve for it to function.

When the connections are made, the battery switch is put in, and the voltage shown is varied by sliding the bridges on the resistance coils. On momentarily depressing the key which connects up the galvanometer, the needle of this instrument will kick one way. The coarse adjustment is then altered until the needle just kicks in the opposite direction on again depressing the key. Further alteration is then carried out with the fine adjust-

ment until, on depressing the galvanometer key, there is no movement in the needle, indicating the null point, where the two E.M.F.'s (that is, the two electromotive forces, the one produced by the combined cell of A and C, each containing a platinum foil, quinhydrone and, respectively, the solution of unknown and known hydrogen ion concentration, and the other from the battery regulated by the coarse and fine adjustment) exactly balance. The E.M.F. produced by the solution, of definite *p*H, is known, and, therefore, that produced by the unknown solution can be found. Such sets are simple and sensitive.

An illustration will make the working clear. Suppose equilibrium is reached—that is, there is no movement one way or the other in the galvanometer on depressing the galvanometer key, when the reading on the millivoltmeter is 140. Then the hydrogen ion concentration is obtained from the following equation (temp. 20° C.):

$$\begin{aligned} p\text{H} &= 3{\cdot}60 + \frac{\text{No. of millivolts}}{58{\cdot}1} \\ &= 3{\cdot}60 + \frac{140}{58{\cdot}1} \\ &= 3{\cdot}60 + 2{\cdot}41 \\ &= 6{\cdot}01 \end{aligned}$$

Colour.—The colours of flours and of cereals generally have attracted considerable attention in the past. For malt, etc., the Lovibond tintometer is normally employed, descriptions of which may be found in brewing books. The Lovibond tintometer does not lend itself to the measurement of the colour of flour. For judging the colour of flours, the only test employed until recently was the Pékar test, in which compressed slabs of flour are placed side by side, dipped into water, and allowed to dry. This is a rough method and can only be used for immediate comparisons. It is not possible to express the results on any numerical scale. The difficulties of judging flour colours are enhanced, as the colours change on storage, and hence flours of standard colours for comparison cannot readily be kept.

Kent-Jones and Herd (*Analyst*, 1927, **52**, 443) have suggested

that useful information on this subject can be obtained, and that the results are capable of being recorded numerically by considering

(*a*) The yellowness due to carotin.

(*b*) The dullness imparted by the presence of finely ground-up offal.

The yellowness can be determined by extracting flour with petrol and matching the tint obtained therein by comparison with potassium chromate solution. The yellowness of flour, which is a rough indication of bleaching, has been a standard test for some time. The A.O.A.C. method advises extracting 20 grms. of flour with 100 c.c. of gasoline (shaking and standing for sixteen hours), and measures the colour against 0·005 per cent. potassium chromate solution in a Schreiner or similar colorimeter. Coleman and Christie (*Cer. Chem.*, 1926, **3**, 84) suggest extracting the flour with gasoline by stirring for fifteen to thirty minutes with a mechanical stirrer, and get similar results to the A.O.A.C. method. In testing wheats in this way, these investigators advise grinding so that at least 75 per cent. will pass through a No. 50 grit gauze.

Kent-Jones and Herd pointed out that many low-grade flours may be bleached white, *i.e.*, that the yellowness due to the carotin may be removed, but that they are still dull and dirty owing to the presence of the offal. The colouring matter of the skins of the wheat grains is said to be xanthophyll, although Simpson (*Milling*, June 22nd, 1929) maintains that it is a flavone; this is not capable of being bleached by the ordinary bleaching reagents used. Further, it is not extractable with petrol. Kent-Jones and Herd, therefore, advise two extractions on flour, using separate portions, firstly, petrol to determine the yellowness, and, secondly, alkaline methyl alcohol to extract the xanthophyll or flavone. A consideration of both these results gives a good idea of the colour of the flour, *i.e.*, whether it is yellow or not, and also whether it is bright and well milled or dull due to offal contamination. Further, the results can be expressed numerically on an empirical scale, as, for instance, by the number of c.c. of standard colouring solution used in water to match a definite quantity of the extract.

A special form of colorimeter is suggested in which the images of both tubes are shown in one split field. The actual procedure and the standard tinting solutions (mixtures of potassium chromate and cobalt nitrate) used are fully described in the reference given above and in *Modern Cereal Chemistry*, pp. 377-385. On the empirical scale suggested, unbleached flours give petrol figures of 12 and over, and bleached ones may be as low as 4 or 5. Similarly, high-grade patent flours give, with the alkaline methyl alcohol extraction, figures as low as 5 or 6, straight-run flours 9 to 10, while low-grades are as high as 11 and over.

This method has been criticised by Visser't Hooft and de Leeuw (*Cer. Chem.*, 1928, **5**, 351), mainly with respect to the gasoline figure, as the results obtained were not in agreement with those found using the Duboseq form of colorimeter. The Dutch investigators suggested that this was due to a change in the hydrogen ion concentration of the solution used for comparison, owing to the varying amounts of standard solution employed. They, therefore, advocated the employment of coloured buffer phosphate solutions as suggested by Jorgensen (*Cer. Chem.*, 1927, **4**, 486). Kent-Jones and Herd (*Cer. Chem.*, 1929, **6**, 33) suggested that the cause of the disagreement was not a hydrogen ion phenomenon, but was due to certain absorption effects of red and yellow light. For comparative work with the Duboseq instrument an acid chromate solution was found to be desirable, as apparently absorption effects of chromate and dichromate ions are more nearly similar than those of chromate and cobalt.

Whatever system is used in such colour tests, it should be remembered that the scales employed are purely arbitrary.

Examination of Flours for Bleachers and Improvers.—There has not been any definite advance recently in our knowledge of the detection and estimation of bleachers and improvers in flour. Foreign mineral matter is thrown down when flour is mixed with carbon tetrachloride, chloroform or any similar organic liquid of about that specific gravity. This can be conveniently done by shaking up the flour with the liquid selected, say, for example, carbon tetrachloride, in a separating funnel. The foreign mineral matter collects at the bottom and can then be

separated and examined. Phosphate can be detected in this sediment by the usual molybdate test.

Bromate is readily found by warming some of the dried material thrown down by carbon tetrachloride in a test-tube with concentrated sulphuric acid, when a distinct smell of bromine is obtained. Ammonium salts are detected by warming with caustic soda, when the freed ammonia can be smelt. Persulphates can similarly be detected and determined by their well-known reactions. There is, however, a simple and quick test for detecting the presence of persulphate in flour. It is not specific for persulphates, but normally the reaction indicates the use of these compounds. The flour is wetted up into a slack dough with water (this is important!); then a 1 per cent. solution of benzidine in alcohol (methylated spirit will do) is poured on. Blue spots rapidly show themselves if the flour has been treated.

Rothenfusser (*Chem. Z.*, 1925, **49**, 285) suggests the following procedure for the detection of benzoyl peroxide, a recently introduced flour bleacher:

A tube, 0·8 cm. bore and 11 cm. long, is marked in three divisions. The flour is introduced up to the first mark by gentle tapping (about 0·7 grm.); petroleum spirit is then added to the second mark (2·5 c.c.), and a thorough mixing effected by vigorous shaking. A 1 per cent. alcoholic solution of *p*-diamino-diphenylamine hydrochloride solution is poured in up to the third mark (1 c.c.) and again the contents are mixed. The tube is allowed to stand, until separation into layers occurs; if a peroxide is present the top layer will have a green colour.

Spencer (*Nat. Assoc. Review*, 1927, p. 632) states that if a benzoyl peroxide treated flour is flattened as for a Pékar test, and a few drops of a freshly prepared 3 per cent. alcoholic guaiacum resin solution be poured on and then immersed in water, it will show up in pale greenish-blue spots. This test does not seem very reliable, however.

The above methods depend upon the reaction of the peroxide. The peroxide, however, decomposes on storage (hence the bleaching action), and these tests may, therefore, fail. It should, however, be possible to detect the minute quantity of benzoic acid left behind in the flour.

Nitrite in flour is detected by the Griess-Illosvay reagent, which is made up as follows :

(i.) 0·5 grm. of sulphanilic acid is dissolved in 150 c.c. of dilute acetic acid.

(ii.) 0·1 grm. of solid *a*-naphthylamine is boiled with 20 c.c. of water, the colourless solution is poured off from the bluish-violet residue, and 150 c.c. of dilute acetic acid are added.

These solutions are then mixed.

The flour is extracted by adding 100 c.c. of tap-water (distilled water, if distilled over a gas flame, contains appreciable quantities of nitrites) to 10 grms. of flour, shaking for about ten minutes, and filtering when the flour has settled. To 50 c.c. of this filtered extract are added 5 c.c. of the freshly prepared Griess-Illosvay reagent. This is then placed in a water bath at 70° C. for about five minutes, when the extracts from bleached flours develop a deep rose colour. A faint red should be disregarded, as most flours —even unbleached—will give this. In fact, it is recommended that the extraction, etc., of the flour should be carried out in the open air and not in the laboratory atmosphere. It is best to work with a known unbleached flour at the same time as a comparison. The parts per million of nitrite may be determined in Nessler glasses by adding to a water extract of known unbleached flour (free from nitrite) definite quantities of a sodium nitrite solution.

This standard sodium nitrite solution can be prepared as follows :

Dissolve 0·405 grm. of pure silver nitrite in boiling distilled water, and add pure sodium chloride until the silver is completely precipitated as silver chloride. Make up to 1 litre and allow the precipitate to settle. Then dilute 100 c.c. of the clear supernatant liquid to 1,000 c.c. One c.c. of the diluted solution is equivalent to 0·00001 grm. of N_2O_3, 0·000012 grm. of NO_2 or 0·000018 grm. of $NaNO_2$.

For example, Swanson, Willard and Fitz (*Kans. Agr. Exp. Sta. Bull.*, 1915, **202**) found moderately bleached fresh flour to contain 2 parts per million of nitrites, and heavily bleached flour to contain 4 parts per million.

Nitrite can also be detected and determined by the use of a weak meta-phenylenediamine solution and a few drops of concentrated

sulphuric acid; if nitrites are present, a brown coloration develops in about fifteen minutes.

Chlorine is not difficult to detect in flour if the quantities used have been about ½ ounce or more per sack. When only traces have been used, the greatest care must be exercised or else the amount of chlorine in the blank will be in excess of that being looked for. It would appear that there is no chlorine in the fat of untreated flour, but, after treatment with chlorine, traces are always found. If flour is roughly extracted with ether and the ether removed, the fat remains behind. If a piece of copper gauze, preferably on a platinum wire, is oxidised in a flame tip until no green colour shows, and then moistened with the fat, the green (due to the chlorine) can readily be detected on again putting the gauze into the flame.

For an actual determination a known quantity of the flour, say 50 to 100 grms., is extracted with ether, filtered through a dry filter paper and evaporated to dryness with 5 c.c. of alcoholic sodium hydroxide solution. This is then carefully charred at low redness, and the char twice extracted with 20 c.c. (1 : 20) nitric acid. The ashing is then completed, the final ash being dissolved in (1 : 20) nitric acid. The collected filtered nitric acid washings and extracts are then neutralised with dry calcium carbonate, 5 c.c. of potassium chromate added as an indicator, and the liquid titrated against standard silver nitrate. A blank determination on a known untreated flour should be carried out at the same time.

Alternatively, the Volhard method may be used, in which excess of silver nitrate is added, and this is titrated with ammonium thiocyanate, the usual ferric indicator being used.

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CHAPTER VII

MILK AND MILK PRODUCTS

By G. D. Elsdon, B.Sc., F.I.C.

Examination of Milk: *Chemical Methods:* Mineral Matter · Fat · Lactose · Acidity and *p*H Value. *Physical Methods:* The Freezing-point Method · Refraction of the Serum · Other Physical Methods. *Miscellaneous Tests:* Detection of Annatto · Routine Examination of Milk. Examination of Cream · Condensed Milk · Dried Milk · Examination of Butter: Composition of Butter Fat · Examination of Butter Fat.

The analytical examination of milk may be required for three, or possibly four, purposes. These are the prosecution of purely scientific research, the compilation of statistics, the detection of adulteration, and the control in production or manufacturing utilisation of milk products. These purposes are intimately connected, and if, in the present sketch of the position of things to-day, the third seems to have received an undue share of notice, it is only because this is the most difficult of all, and that anything that is included under this head will of necessity be included under the others. Most of the methods which have been proposed for the examination of milk products are modifications of those which have been applied to milk itself. It is proposed, therefore, to deal with milk first, and afterwards to indicate the applications of the various methods suggested to milk products. The processes which have been adopted have been both chemical and physical. Physical processes seem to be particularly well adapted to the examination of natural articles and have come into increasing use in recent years; they will, therefore, have special and separate mention.

THE EXAMINATION OF MILK

Part I.—Chemical Methods

Milk being a natural mixture of fat, carbohydrates, proteins and mineral matter, it will be obvious that the problem of its analytical

examination should first be attacked along the classical lines for each of these classes of substances. The history of milk analysis is the history of the difficulties which have been encountered in such applications, and in the separation of this natural mixture into its constituents. Milk has always been regarded as a more or less ideal emulsion, and the perfection of this emulsion is a measure of the difficulties which have been encountered in its separation. Each constituent will be considered under a separate heading.

Mineral Matter.—The total amount of mineral matter has mostly been determined by means of ignition. This is not, of course, an absolute method of determination, as there are a number of possible sources of error. It is, nevertheless, a method which gives comparative results which are sufficiently near the truth for all ordinary purposes, when the ignition is done with care. The temperature should be as low as possible, certainly not more than a very dull red heat, and the operation must be continued until the ash is absolutely white and shows no black specks on treating with a little water. In cases where such black specks are noticed the water is allowed to evaporate on the water bath, the dish heated gently until all danger of spurting has passed, and finally ignited at a low temperature. The ash of cow's milk usually varies between 0·70 per cent. and 0·80 per cent., with an average of about 0·75 per cent. G. N. Quam and A. Hellwig (*J. Biol. Chem.*, 1928, **78**, 681 ; *Analyst*, 1928, **53**, 542) state that copper is a natural constituent of milk to the extent of 0·26 to 0·52 mgrm. per litre. G. Bertrand and H. Agulhon (*Bull. Soc. Chim.*, 1913, **13**, 824 ; *Analyst*, 1914, **39**, 123) found in human milk 0·45 of boric acid, in ass's milk 0·55, and in cow's milk 1·1, expressed in mgrms. per litre.

Direct determination of some of the mineral constituents of milk has been attempted with success. Thus C. S. Rothwell (*J. Biol. Chem.*, 1925, **65**, 129 ; *Analyst*, 1925, **50**, 562) determines calcium by direct precipitation with ammonium oxalate, the fat being removed by washing the precipitate with a mixture of ether and ammonium hydroxide. The same worker has applied this as a micro method, especially to human milk (*J. Biol. Chem.*,

1927, **75**, 23 ; *Analyst*, 1927, **52**, 716). One c.c. of the milk is placed in a 15-c.c. tapered centrifuge tube, and 2 c.c. of a 10 per cent. solution of sodium chloride and 1 c.c. of a saturated solution of ammonium oxalate added. The liquid is mixed and left for about an hour. The tube is then centrifuged, the supernatant milk and cream decanted, and the residue washed once with about 0·5 c.c. of ether and 2 c.c. of ammonia solution (2 c.c. of conc. ammonia to 100 c.c. of water). The washing is repeated with 2 c.c. of the dilute ammonia solution, care being taken not to disturb the precipitate while washing. The precipitated calcium oxalate is titrated with 0·01*N* potassium permanganate in the usual manner.

Chlorine : Chlorides may also be determined directly after treating the milk in a suitable manner. Nitric acid may be used (J. Werder, *Mitt. Lebensm. Hyg.*, 1921, **12**, 37 ; *Analyst*, 1921, **46**, 498. J. Drost, *Z. Unters. Nahr. Genüssm.*, 1925, **49**, 332 ; *Analyst*, 1925, **50**, 624), or an acetic acid solution of picric acid. A. D. Husband and W. Godden (*Biochem. J.*, 1927, **21**, 259 ; *Analyst*, 1927, **52**, 288) carry out the latter method, which is due to Austin and Van Slyke, in the following way : Forty c.c. of 1·2 per cent. picric acid solution, containing 2 c.c. of glacial acetic acid per litre, are added to 20 c.c. of milk, and the whole filtered after standing for ten minutes. Thirty c.c. of the clear filtrate are treated with 10 c.c. of *N*/10 silver nitrate solution, the mixture shaken, again filtered, and 20 c.c titrated by Volhard's method.

L. Barthe and E. Dufilho (*Ann. Falsif.*, 1927, **20**, 88 ; *Analyst*, 1927, **52**, 287) found that the percentage of combined chlorine in cow's milk does not exceed 0·15 per cent., and that this is not increased by the administration on two successive occasions of 25 and 50 grms. of salt. The same authors have found that the amount of sodium in cow's milk does not exceed 0·05 per cent.

L. L. Van Slyke and J. C. Baker (*J. Biol. Chem.*, 1919, **40**, 335 ; *Analyst*, 1920, **45**, 139) determine carbon dioxide and carbonates in a Van Slyke carbon dioxide apparatus (*J. Biol. Chem.*, 1917, **30**, 347 ; *J. Soc. Chem. Ind.* 1917, **36**, 944), after treating with lactic acid. The normal value found was about 10 per cent. by volume of carbon dioxide. On pasteurisation the amount falls,

and the suggestion is made that this determination might be used to distinguish heated milk from normal milk.

Fat.—It is quite impossible to remove the whole of the fat from milk by shaking with immiscible fat solvents. The original method for the determination of fat, due to Wanklyn, was soon found to be inaccurate, and, as a result, various methods and modifications have been proposed. All the suggestions have one point in common, viz., that they endeavour to treat the milk in such a manner that the difficulties which arise from the mucoid layer, which apparently surrounds the fat globules, shall be overcome. Of the various methods which have been proposed, the more satisfactory are probably those due to Adams, Bell, Röse-Gottlieb and Werner-Schmid. All these processes are, in skilled hands, capable of giving equally accurate results, when all precautions are taken and appropriate corrections made. Latterly, however, the Rose-Gottlieb method seems to have come into general favour. It can be wholly recommended. The latest method of carrying out this process is that given under the determination of fat in condensed milk on p. 256, 10 grms. of milk being used in place of the 2 grms. of condensed milk diluted with water.

Proteins.—The determination of the proteins may be regarded from two points of view. It may be required to determine the total proteins present, which may be carried out by the ordinary Kjeldahl method (nitrogen × 6·38 = proteins), or by some precipitation method, or a more complete examination of the proteins may be required, with separation of the casein, albumin, globulin, etc. This latter is by no means easy of accomplishment—in fact, it can be said with some certainty that no really satisfactory method is at present available. The somewhat wide variations which are to be found in the published figures for, say, albumin are doubtless to be attributed, at least in part, to this factor.

This subject is at present under investigation at the National Institute for Research in Dairying at Reading, and as a result of this work it is hoped that more satisfactory methods will become available.

H. C. Waterman (*J. Assoc. Off. Agric. Chem.*, 1927, **10**, 259;

Analyst, 1927, **52**, 548) has suggested precipitation of the casein at approximately the iso-electric point, and avoids the tedious washing of precipitates by the determination of total nitrogen in the milk and in the serum after precipitation.

My experience would suggest that considerably more attention should be given to the technique of the Kjeldahl test than is usually the case. The digestion flask should be allowed to rest on a support of asbestos in which a circular hole has been cut that will accurately fit the flask. In no circumstances should the flame be allowed to come up between the asbestos and the flask, and the acid layer should rise above the level of the asbestos. Furthermore, the acid should not be allowed to boil, the amount of acid should always be considerably in excess of that required to produce potassium bisulphate, and the heating should be continued for at least one hour after the colour has ceased to diminish. Quite recently cases have come under my notice in which the figures given by different persons on the same sample have varied from 7·8 per cent. to 9·4 per cent., so that it is obvious that too great care cannot be given to this process.

Lactose. One of the chief difficulties which are encountered in the determination of lactose in milk is due to the necessity of preparing a clear serum, which in some cases, as for polarimetric work, must be free from nitrogen. The method of Lane and Eynon (*J. Soc. Chem. Ind.*, 1923, **42**, 32*t* : *cf.* p. 27) may be used directly on the diluted milk, but difficulties will arise from bumping. With care, however, very fair results can be obtained by this method. It has the great advantage of simplicity and rapidity.

The preparation of a clear serum can be carried out in many ways. The use of dialysed iron has been suggested by Hurst (*Analyst*, 1925, **50**, 138). Ten c.c. of milk slightly diluted with water are precipitated with 12 c.c. of dialysed iron (B.D.H.). The mixture is diluted to a definite bulk, well shaken, and filtered through paper. This is an elegant method which gives a beautifully bright serum, but it cannot be used for polarimetric methods, on account of the considerable dilution necessary and the fact that optically active nitrogen compounds are left in solution. It may

be used with the Lane and Eynon method or the iodine method (pp. 33 *et seq.*).

It is not easy to find the volume of the precipitate. The volume of precipitated fat and protein in c.c. is usually taken as F × 1·08 + P × 0·74, where F and P are the weights of fat and protein in grms., respectively, involved. There is, however, a further correction needed, due to the volume of the precipitant. This, of course, varies with the precipitant used. At the moment it is only possible to say exactly what this volume is in the case of the zinc ferrocyanide precipitant. Further details on these points are given in the section on condensed milk on p. 257.

Several micro and semi-micro methods have been suggested, some of which are carried out directly on the milk. O. Folin and W. Denis (*J. Biol. Chem.*, 1918, **33**, 521; *Analyst*, 1918, **43**, 294) take 2·8 to 3·4 c.c. of a diluted milk (25 c.c. to 100 c.c.), 5 c.c. of copper sulphate solution (60 grms. of crystallised copper sulphate and 4 c.c. of conc. sulphuric acid per litre) and 4 to 5 grms. of a dry salt mixture (100 grms. of disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$), 60 grms. of sodium carbonate, and 30 grms. of sodium or potassium thiocyanate) and boil for four minutes, adding a small pebble to prevent bumping. The diluted milk is then added a few drops at a time, with boiling for one minute each time, until the liquid is just colourless. The total boiling period should be from five to seven minutes. The volume of diluted milk required contains 40·4 mgrms. of lactose. A colorimetric micro-Fehling method is described by G. Fontès and L. Thivolle (*Ann. Chim. anal.*, 1922, **11**, 341; *Analyst*, 1923, **48**, 29).

An oxidation method is described by F. T. Adriano (*Philippine J. Sci.*, 1920, **17**, 213; *Analyst*, 1921, **46**, 137).

In this, 25 c.c. of milk are diluted to 400 c.c., 10 c.c. of Fehling's copper sulphate solution added, the whole nearly neutralised with sodium hydroxide solution diluted to 500 c.c., and filtered. Ten c.c. of the filtrate are mixed with 50 c.c. of N/10 permanganate and 25 c.c. of 0·848 per cent. sodium carbonate solution, the mixture diluted to 100 c.c. and heated, the temperature being raised to 95° in two minutes and maintained for a further two minutes at the same temperature. Twenty-five c.c. of 30 per cent. sulphuric acid are added and N/10 oxalic acid until the solution is clear; the excess of oxalic acid is then

titrated with $N/10$ permanganate. A table of lactose factors is given. For the actual determination of sugars see CHAPTER I., pp. 21 *et seq.*

The Acidity and *p*H Value of Milk. S. W. Clausen (*J. Biol. Chem.*, 1922, **52**, 263; *Analyst*, 1922, **47**, 363) determines small quantities of lactic acid by removing albumin by tungstic acid and extracting the lactic acid with ether. The lactic acid is oxidised with permanganate, and the aldehyde so formed is determined by passing it into sodium bisulphite and titrating with iodine.

Certain difficulties are encountered in the determination of the *p*H value of milk. These have been overcome to a considerable extent by V. Lester (*J. Agric. Sci.*, 1924, **14**, 635; *Analyst*, 1925, **50**, 37) by the use of the quinhydrone electrode.

P. F. Sharp and T. J. McInerney (*J. Biol. Chem.*, 1926, **70**, 729; *Analyst*, 1927, **52**, 36) determine the *p*H colorimetrically by diluting the milk 1 to 20 and using a correction factor drawn from a curve. The results are correct to about 0·1. The same authors have a paper on the relationship of *p*H to titratable acidity of milk (*J. Biol. Chem.*, 1927, **75**, 177; *Analyst*, 1927, **52**, 715) in which they say that a relation between the two can be found, so that the *p*H can be determined from the titratable acidity, with an average error of ± 0·06, provided that the titratable acidity is greater than 0·10 per cent., expressed as lactic acid. L. L. Van Slyke and J. C. Baker (*J. Biol. Chem.*, 1919, **40**, 335; *Analyst*, 1920, **45**, 139) found that in 80 per cent. of 300 fresh milks the *p*H was under 6·76, the range being 6·50 to 7·20. J. H. Coste and E. T. Shelbourn (*Analyst*, 1919, **44**, 158) show that the determination of the electrical conductivity of milk is of no value in the detection of added water.

Part II.—Physical Methods

Whilst the progress made in recent years in the chemical examination of milk has been mostly in matters of detail and has not been particularly striking, considerable attention has been given to physical methods. These are mainly of value in determining whether a particular milk is in its natural state or if it has been diluted with water. The fact on which the utility of such methods is based is that the osmotic pressure of cow's blood is the same,

or practically so, for different animals, and that the osmotic pressure of milk bears a more or less constant relationship to this. It follows, therefore, that the osmotic pressure of milk will be practically constant. The accurate determination of osmotic pressure directly is out of the question, so that this is carried out indirectly by means of a determination of the freezing point.

The Freezing-point Method.—There seems to be little doubt that the freezing-point method is the most certain which we have for the determination of added water in milk. By using a simple technique, a practical observer may carry out several determinations in an hour, so that the method is rapid as well as accurate. Various methods have been suggested by various workers, but the simplest method, and one which is apparently capable of giving useful results, is that of Andrews (*Analyst*, 1929, **54**, 210). The inner tube of an ordinary Beckmann's apparatus is about half-filled with the milk under examination, and the thermometer and stirrer fitted into position. This tube is then placed in a strong freezing mixture of ice and salt, and the whole stirred until the thermometer, having fallen, commences to rise. The tube is then placed in the air jacket of the Beckmann apparatus surrounded by a mild freezing-mixture of ice and salt. The stirring is continued until the temperature remains constant, and this point is taken as the freezing point of milk. The freezing point of water is obtained by similar methods, and the difference is taken as the depression of the freezing point due to the milk.

The use of ice and salt in the Beckmann apparatus, although requiring no special apparatus, is not easy to control, and various pieces of special apparatus have been devised for the purpose. The one that has been most widely used is that of Hortvet (*J. Ind. Eng. Chem.*, 1921, **13**, 198; *J. Soc. Chem. Ind.*, 1921, **40**, 274A), this method having been adopted as official by the American A.O.A.C. (*Methods of Analysis*, 2nd Ed., p. 265). It is based upon the method of Monier-Williams (*Report to Local Government Board, Food Reports*, No. 22, 1914). By means of this apparatus, it is possible to carry out accurate determinations in a compara-

tively short time. No ice is used, the freezing being brought about by the evaporation of ether. The determinations are made under standard conditions, and no corrections are made for the usual sources of error in freezing-point determinations. The depressions thus found are probably a little too high, but, as they are strictly comparable one with another, no error is introduced from this source.

A very large number of observers, working on thousands of samples of milk, have established the fact that the depression of the freezing point of genuine milk is never less than 0·534°, when determined in the Hortvet apparatus, and that milks of unusual composition, showing a proportion of solids-not-fat abnormally low, are more likely to give greater than a less depression. Sour milks give abnormally high depressions (*cf.* A. J. Parker and L. S. Spackman; *Analyst*, 1929, **54**, 217).

The Refraction of the Serum.—The possibility that the refraction of milk serum might give equally useful results has been considered by many workers. The results will depend, to a certain extent, on the manner adopted to prepare the serum. The more usual method is to mix a convenient quantity of milk (say, 20 to 40 c.c.) with a quarter of its volume of a copper sulphate solution having an immersion refractometer reading of 36·0 at 20° (about 71·5 grms. of crystallised copper sulphate per litre) and filter. The milk must be fresh, when a normal milk will give a reading of from 37·0 to 39·0, average about 38·3.

The reading will depend, of course, upon the amount and the type of the other constituents, so that, in practice, the refraction is found to be roughly proportional to the lactose, or to the solids-not-fat. The refraction, then, has not been found to be particularly valuable for the purpose of detecting added water, and is certainly very inferior to the freezing-point method (*cf.* Elsdon and Stubbs, *Analyst*, 1927, **52**, 193; 1928, **53**, 150; 1929, **54**, 318; *Chem. & Ind.*, 1928, **47**, 1145; *Chemical Age*, 1929, **20**, 271).

Other Physical Methods.—M. J. N. Schuursma (*Chem. Weekbl.*, 1924, 365; *Analyst*, 1924, **49**, 487) suggests the use of hæmolysis for the detection of added water. For this purpose, portions of

10 c.c. of normal milk are pipetted into dry test-tubes and distilled water added in amounts from 1 c.c. upwards, 5 drops of fresh ox blood being added to each, the solution well mixed, and, after standing for fifteen minutes, centrifuged for at least twenty minutes. The dilution at which haemolysis begins is indicated by the first appearance of a red coloration in the supernatant liquid. The milk to be tested is now diluted in the same way, and the point at which haemolysis begins should correspond to that already determined in the blank. No data are available with which to form a judgment as to the value of this method, but it would not appear likely to supersede the determination of the freezing point.

An attempt has been made by several observers to calculate a figure that would be proportional to the osmotic pressure, and therefore constant, from chemical composition. This method seems to have been originated by L. Mathieu and L. Ferré (*Ann. Falsif.*, 1914, **7**, 12 ; *J. Soc. Chem. Ind.*, 1914, **33**, 214). P. Post (*Pharm. Weekbl.*, 1926, **63**, 983 ; *Brit. Chem. Abst.*, 1926, **1B**, 846) determines the lactose, chlorine and acidity. From the first and last the depression of the freezing point due to lactose is found, and by adding that due to sodium chloride the "cryolac number" is obtained : this is stated to bear a constant ratio to the total depression of the freezing point. The "cryolac number" for unadulterated milk is given as 425, but J. Fiehe and W. Kordatzki (*Z. Unters. Lebensm.*, 1928, **55**, 251 ; *Brit. Chem. Abst.*, 1928, **3B**, 687) find this to vary between 393 and 435, with a mean of 413, and that 75 per cent. of the total freezing-point depression of the milk is accounted for in this way. This method has no advantage over the ordinary determination of solids-not-fat and cannot replace the determination of the freezing point.

Part III.—Miscellaneous Tests

The Determination of Citric Acid.—D. W. Stewart (*Analyst*, 1924, **49**, 465) has found the citric acid content of fresh milk to be 0·158 per cent. of anhydrous citric acid and 1·16 per cent. in the case of dried milk. He prefers the pentabromacetone method,

which is carried out as follows (R. Kunz, *Archiv. Chem. Microsk.*, 1915, **8**, 120 ; *Analyst*, 1916, **41**, 378) :

Fifty c.c. of milk are treated with 20 c.c. of 50 per cent. sulphuric acid, 2 c.c. of 40 per cent. potassium bromide solution, and 20 c.c. of 5 per cent. phosphotungstic acid solution, diluted to 20 c.c., shaken and filtered. To 150 c.c. of the filtrate are added 25 c.c. of freshly prepared saturated hydrobromic acid solution, heated at 50° for five minutes, and then treated with 10 c.c. of 5 per cent. potassium permanganate solution, added gradually while the mixture is stirred. Any traces of manganese dioxide are removed by the addition of a drop of ferrous sulphate solution containing sulphuric acid. The pentabromacetone is filtered off and weighed.

Kunz found about 0·19 per cent. in ordinary milk, but G. C. Supplee and B. Bellis (*J. Biol. Chem.*, 1921, **48**, 453 ; *Analyst*, 1922, **47**, 24) found about 0·14 per cent.

The Detection of Nitrates.—M. E. Pozzi-Escot (*Bull. Soc. Chim.*, 1924, **35**, 72 ; *Analyst*, 1924, **49**, 233) curdles the milk with sulphuric acid, neutralises the whey with ammonia, evaporates to a syrup, and triturates the cold residue with cold, slightly diluted, sulphuric acid and a little ether. The ether dissolves the nitrate as nitric acid. It is decanted, neutralised with ammonia, evaporated at a low temperature, and the residue tested for nitrate. A. F. Lerrigo (*Soc. Public Analysts'* Meeting, April 2, 1930) recommends the use of a routine test for nitrates as a means of detecting added water in milk.

The Detection of Annatto.—A. G. Gardiner (*Analyst*, 1925, **50**, 549) coagulates 25 c.c. of milk at 50° with 0·2 c.c. of glacial acetic acid. The curd is collected on a Buchner funnel, returned to the original flask, shaken with 75 c.c. of ether and allowed to stand overnight. The ethereal solution is evaporated to dryness, made alkaline by adding 6 c.c. of *N*/10 sodium hydroxide solution, stirred thoroughly and transferred to a wet 9-cm. paper. When all the liquid has passed through and only fat remains, the paper is opened out, placed on a clock glass, washed with a stream of hot water and allowed to dry in the air. A pink colour indicates annatto. I have found this test, which was independently worked out by J. R. Stubbs (private communication), to work well.

The Routine Examination of Milk. The Mojonnier tester is described by L. H. Lampitt, E. B. Hughes and M. Bogod (*Analyst*, 1924, **49**, 413). By this means, routine determination of total solids and fat, of high accuracy, can be carried out in a very short time. The total solids are determined by evaporation on a hot plate in a vacuum, and the fat is determined by a modified Rose-Gottlieb method. Various points in connection with the mechanical determination of fat are considered by F. E. Day and M. Grimes (*Analyst*, 1918, **43**, 123), H. D. Richmond (*Ibid.*, 1918, **43**, 405), F. E. Day (*Ibid.*, 1920, **45**, 411), T. F. and C. O. Harvey (*Ibid.*, 1923, **48**, 213) and B. J. Smit (*Ibid.*, 1923, **48**, 477).

THE EXAMINATION OF CREAM

It has been pointed out by A. F. Lerrigo (*Analyst*, 1928, **53**, 488) that many commercial samples of cream contain added water. The same author (*Ibid.*, 1928, **53**, 335) detects glycerin in cream by the loss in weight undergone by the dried solids on further heating, fuming of the solids when heated, and excessive browning, but finds that 5 per cent. of glycerin does not appear to exercise any preservative effect.

THE EXAMINATION OF CONDENSED MILK

Fat and Total Solids. R. W. Sutton (*Analyst*, 1925, **50**, 17) determines fat in condensed milk by precipitating with copper sulphate solution and treating the precipitate as in the Werner-Schmid process. J. McCrae (*Ibid.*, p. 236) avoids filtration by carrying out the precipitation in a centrifuge tube. If the precipitation is carried out in a Leffman and Beam bottle, the fat can be determined mechanically.

The standard processes suggested by the Milk Products Sub-Committee of the Standing Committee on Uniformity of Analytical Methods, Society of Public Analysts (*Analyst*, 1927, **52**, 402) are given below. They are not necessarily the simplest nor the shortest, but they are capable of giving concordant results in the hands of experienced workers. In the case of total solids,

great attention should be given to desiccation and to the temperature of the oven.

Total Solids. *Preparation of the Support.* Select for use sand which passes a 30-mesh and is retained by a 90-mesh sieve. Heat a convenient quantity of this sand with strong hydrochloric acid to remove oxide of iron, etc.; decant; repeat the digestion till the acid liquor is nearly colourless; wash, once with dilute hydrochloric acid, and then thoroughly with distilled water; dry and ignite.

The sand thus prepared should be tested for suitability as follows: Dry a portion at 98° to 100° C. and weigh; moisten with distilled water and subsequently dry again at 98° to 100° C. There should be no difference between the two weights.

Dishes. These should be of metal (aluminium or nickel is suitable), with readily removable but close-fitting lids; a suitable size is of diameter about 3 inches and depth about 1 inch.

Procedure. (1) *Sweetened Condensed Milk.* Place about 25 grms. of the prepared sand and a short glass stirring rod in the dish and dry to constant weight in an oven at 98° to 100° C., the lid being removed while drying and replaced before removing the dish from the oven. Allow the dish to remain for forty-five minutes in the desiccator before weighing.

Tilt the sand to one side of the dish; place on the clear space about 1·5 grms. of the well-mixed sample and weigh rapidly. Add 5 ml. of water to the milk and mix these; then mix the diluted milk thoroughly with the sand by means of the rod.

Place the dish on a rapidly boiling water bath for twenty minutes, carefully stirring during the earlier period. Transfer the dish, with rod and cover, to a well-ventilated oven at 98° to 100° C., as recorded by a thermometer in the air immediately above the dish. After one and a half hours, cover the dish and place in the desiccator for forty-five minutes; weigh; return the dish to the oven, and heat for one hour with lid removed; remove and weigh as before; repeat this process until the loss of weight between successive weighings does not exceed 0·0005 grm.

(In a satisfactory determination it is generally found that the loss between the second and third weighings does not exceed 0·0005 grm.)

(2) *Unsweetened Condensed Milk.* Weigh out 3 grms. of condensed milk and use 3 ml. of water; otherwise proceed as in (1).

Fat. Determine the fat according to the following modification of the Röse-Gottlieb method:

Reagents.—Concentrated ammonia solution, nominal 0·880.

Alcohol or industrial methylated spirit, 95 per cent. by volume.

Ether (methylated), sp. gr., 0·720.

Petroleum spirit, boiling between 40° C. and 60° C.

These reagents should leave no appreciable residue on evaporation.

Procedure.—Transfer to a suitable apparatus from 2 to 2·5 grms., accurately weighed, of the well-mixed sample; add 8 ml. of warm water and mix well; cool; add 1 ml. of concentrated ammonia solution, mix, add 10 ml. of alcohol and again mix. Add 25 ml. of ether and shake vigorously for one minute; add 25 ml. of petroleum spirit and again shake vigorously for thirty seconds. Allow the liquids to stand for not less than half an hour, until the ethereal layer is perfectly clear, or centrifuge at a low speed. Transfer the ethereal layer to a suitable flask. To the milk residue add 5 ml. of ether, and transfer without further shaking; repeat this operation in the same manner with a further 5 ml. of ether. Add 0·5 ml. of alcohol, and repeat the extraction with 25 ml. of ether and 25 ml. of petroleum spirit, as before, shaking vigorously for one minute after the addition of the ether and for thirty seconds after the addition of the petroleum spirit. As before, allow the ethereal layer to separate completely and transfer to the flask. Repeat the extraction once more with alcohol, ether and petroleum spirit in the same manner.

Cautiously distil the solvents from the flask and dry the residual fat at 98° to 100° C. to constant weight, taking the ordinary precautions to remove all traces of volatile solvent.

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation. Finally, dry the flask at 98° to 100° C. The difference in weights before and after the petroleum spirit extractions is the weight of fat contained in the quantity of condensed milk taken.

Make a blank determination, using the specified quantities of reagents and distilled water in place of the milk, and deduct the figure found, if any, from the weight of fat obtained.

Sugars.—As in milk, one of the great difficulties in the determination of sugars (either lactose or added sucrose) in condensed milk is the volume of the precipitate which is produced on obtaining a serum. This precipitate is due to the fat and protein, together with more or less of the precipitant used. The correction which has been used up to now is, volume of precipitate in c.c. = per cent. of fat × 1·08 + per cent. of protein × 0·74, but this does not take into account the volume of the precipitant which comes down with the fat and protein. In certain cases, *e.g.*, when the precipitant is phosphotungstic acid, dialysed iron or zinc ferrocyanide, this volume is material and must be allowed for.

P. Honegger (*Analyst*, 1926, **51**, 496) makes a modification of Revis and Payne's method, using acid mercuric nitrate, and adopting an empirical inversion factor. This method is somewhat adversely criticised by H. D. Richmond (*Analyst*, 1927, **52**, 525). Other work on the subject has been carried out by G. G. Jorgensen (private pamphlet; *Analyst*, 1925, **50**, 143), and by G. K. Scheringa (*Pharm. Weekbl.*, 1925, **62**, 1034; *Analyst*, 1925, **50**, 625).

G. W. Monier-Williams (*Analyst*, 1928, **53**, 569) has attempted to get over the difficulty introduced by the volume of the precipitate by determination of the total solids of the serum as well as those of the milk. He proceeds in the following way:

Approximately 350 grms. of the milk and sucrose mixture, weighed to 0·1 grm., are treated with 1·5 grm. of (powdered) citric acid, which coagulates the casein. The coagulated milk is shaken, and to it is added, in small portions at a time, a mixture of 9 grms. of phosphotungstic acid and 45 grms. of dry sand, the two having been previously ground together in a mortar. If more or less of the milk and sucrose mixture is taken, the amounts of citric and phosphotungstic acids are varied accordingly. The quantity of citric acid added should be that necessary to coagulate the casein, and of phosphotungstic acid that necessary to effect complete precipitation of proteins while leaving a slight excess of phosphotungstic acid in solution. The liquid is thoroughly shaken after each addition of phosphotungstic acid and sand. The object of the sand is to effect fine sub-division of the phosphotungstic acid crystals, and to assist in breaking up the curd during the subsequent shaking.

The liquid is then filtered through a dry, folded filter, the first runnings of the filtrate being rejected. A total solids determination is carried out on the filtrate.

Fifty c.c. of the clear filtrate are measured into a dry weighed 100-c.c. measuring flask, and the weight ascertained. From the total solids determination the actual weight of water in this 50 c.c. is calculated (y in the formula below). To the 50 c.c. of filtrate are added 2·675 grms. of dry ammonium chloride, and the contents of the flask made up to the mark, allowed to stand for one hour, and polarised in a 200-mm. water-jacketed tube. The temperature of the liquid should be between 18° and 22° C., and should be recorded to within 0·2° C. by means of a small thermometer placed in the opening of the jacketed tube. The thermometer is allowed to remain in the liquid until the temperature indicated is constant, and is then withdrawn immediately

before the readings are taken. The mean of the readings is represented by A in the formula below.

A second portion of 50 c.c. of the filtrate is measured into the same 100-c.c. flask with the same pipette, and exactly 10 c.c. of 5*N* hydrochloric acid are added. The mixture is placed in a water-bath at 60° C. for twelve minutes, the flask being agitated for the first four minutes to promote rapid heating. It is then withdrawn and cooled, and 10 c.c. of 5*N* ammonia solution added slowly, with shaking, from a burette. The ammonia solution need not be exactly 5*N*, but its strength in terms of 5*N* hydrochloric acid must be known, and an amount added just sufficient to neutralise the hydrochloric acid used. The contents of the flask are again cooled, if warm, and made up to the mark.

If the solution is cloudy it must be filtered through a dry filter before polarisation, the first runnings being rejected. It is then allowed to stand for one hour and polarised in the same way and at as nearly as possible the same temperature as the uninverted solution. Accurate measurement of the temperature is particularly important in this case.

The mean of the readings is represented by B in the formula below, and A − B is the change of rotation on inversion of the filtrate as diluted for polarisation.

The percentage of sucrose in the milk and sucrose mixture is then given by the formula :

$$\frac{\mathrm{A} - \mathrm{B}}{87{\cdot}9 + 0{\cdot}06c - 0{\cdot}3(t - 20)} \times \frac{100}{2} \times \frac{x}{y},$$

where c is the percentage concentration of total sugars in the inverted solution, as diluted for polarisation ; t is the temperature of invert polarisation ; A is the direct reading of the filtrate as diluted for polarisation ; B is the invert reading of the filtrate as diluted for polarisation ; x is the percentage of water by weight in the diluted condensed milk or milk and sucrose mixture ; y is the weight of water in the 50 c.c. of filtrate taken for polarisation.

This method is ingenious, and may be recommended as one likely to give accurate results. The main objections are the large amount of manipulation which is required, and the fact that the state of hydration of the precipitant and of the total solids is somewhat uncertain.

For those who prefer to use a volume correction and in all cases of dispute, the following process recommended by the Milk Products Sub-Committee in their Report to the Standing Committee

on Uniformity of Analytical Methods of the Society of Public Analysts should be adopted.

Society of Public Analysts' Method. *Reagents.* Zinc acetate solution: 21·9 grms. of crystallised zinc acetate, $Zn(C_2H_3O_2)_2.2H_2O$, and 3 ml. of glacial acetic acid, in water, made up to 100 ml.

Potassium ferrocyanide solution: 10·6 grms. of crystallised potassium ferrocyanide in water made up to 100 ml.

Concentrated hydrochloric acid solution = 6·34 times normal.

Concentrated ammonia solution, nominal 0·880.

Dilute ammonia solution, 10 ml. of concentrated ammonia solution diluted with water to 100 ml.

Dilute acetic acid solution approximately equivalent to the dilute ammonia solution.

Apparatus. The instrument used for measuring the optical rotation may be either a polarimeter or a saccharimeter, using, for the polarimeter, sodium light, or the green line of the mercury spectrum separated by means of a prism or by the use of a special Wratten screen No. 77a, and for the saccharimeter white light from an incandescent electric lamp after passing through 15 mm. of a 6 per cent. solution of potassium bichromate.

Tubes, of not less than 2 dm., exactly calibrated for length.

Flasks and pipettes accurately calibrated in ml. at 20° C.

A standardised thermometer, reading to 0·1° C.

Procedure. Transfer to a 100-ml. beaker an accurately weighed quantity, approximately 40 grms., of the well-mixed sample: add 50 ml. of hot, distilled water (80° to 90° C.), mix, transfer to a 200-ml. measuring flask, washing in with successive quantities of distilled water at 60° C., until the total volume is from 120 to 150 ml. Mix, cool to air temperature, and then add 5 ml. of the dilute ammonia solution. Again mix, and allow to stand for fifteen minutes. Add a sufficient quantity of the dilute acetic acid solution to neutralise the ammonia added (the exact equivalent is determined beforehand by titration), and again mix. Add, with gentle mixing, 12·5 ml. of zinc acetate solution and mix, followed in the same manner by 12·5 ml. of potassium ferrocyanide solution. Bring the contents of the flask to 20° C. and add distilled water at 20° C. up to the 200-ml. mark.

Up to this stage all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles, and, with the same object in view, all mixings should be made by rotation of the flask rather than by shaking. If bubbles are found to be present before completion of dilution to 200 ml., their removal can be assisted by temporary attachment of the flask to a vacuum pump, and rotation of the flask.

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for a few minutes and then filter through a dry filter paper, rejecting the first 25 ml. of filtrate.

Direct Polarisation.—Determine the rotation of the filtrate at 20° C.

Inversion.—Pipette 40 ml. of the filtrate obtained as above into a 50-ml. flask; add 6 ml. of 6·34 normal hydrochloric acid. Immerse for twelve minutes the entire bulb of the flask in a water-bath maintained at 60° C., mixing by rotatory movement during the first three minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, dilute to 50 ml. at 20° C. with distilled water, mix and allow to stand for one hour.

Invert Polarisation.—Determine the rotation at 20° C.

Calculation:

W = weight of sample taken in grms.
F = percentage of fat in the sample.
P = percentage of protein (N × 6·38) in the sample.
V = volume to which the sample is diluted before filtration.
v = correction in ml. for volume of precipitate produced during clarification.
D = observed direct polarimeter reading.
I = observed invert polarimeter reading.
l = length in dm. of polarimeter tube.
Q = inversion divisor factor.

Then $v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 1{\cdot}55)]$,

and the percentage of sucrose in the sample

$$\left(D - I\right) \times \frac{V}{Q} \times \frac{V}{l \times W}$$

The value to be used for Q depends upon the source of light, the type of polarimeter and (if the reading is not taken exactly at 20° C.) the temperature used. At 20° C. the value of Q under the conditions of this method is:

For sodium light . . .	0·8825
For mercury green light . .	1·0392
For white light (*j*) . . .	2·5490

(*j*) International Sugar Scale.

When the invert reading is not taken exactly at 20° C., or when the concentration of total sugars in the solution is very different from 9 per cent., the value of Q may be corrected by means of the following equations, where C is the total concentration of the sugars in the solution

as polarised and T is the temperature at which the invert polarisation is read.

For sodium light	$Q = 0{\cdot}8825 + 0{\cdot}0006\,(C-9) - 0{\cdot}0033\,(T-20)$
For mercury green light	$Q = 1{\cdot}0392 - 0{\cdot}0007\,(C-9) - 0{\cdot}0039\,(T-20)$
For white light (*j*)	$Q = 2{\cdot}5490 - 0{\cdot}0017\,(C-9) - 0{\cdot}0095\,(T-20)$

(International Sugar Scale.

Condensed Milk Calculations.—In England the sale of condensed milk is controlled by the Public Health (Condensed Milk) Regulations, 1923 and 1927. Under these Regulations Condensed Full Cream Milk and Condensed Skimmed Milk (both sweetened and unsweetened in each case) are both recognised. Receptacles have to be labelled with the number of equivalent pints of fresh milk. Tables to aid in the requisite calculations have been prepared. See E. Hinks, *Analyst*, 1923, **48**, 596; R. E. Essery, *Ibid.*, 1924, **49**, 178; J. F. Liverseege, *Ibid.*, p. 276.

THE EXAMINATION OF DRIED MILK

The examination of dried milk, although one of seeming simplicity, needs to be carried out with a considerable amount of care. The following points should be taken into serious account:

Moisture.—N. Schoorl and S. C. L. Gerritzen (*Pharm. Weekbl.*, 1921, **58**, 370; *Analyst*, 1921, **46**, 241) found that milk dried at 95° or 100° reaches constancy in three to four hours, but that the figure thus obtained is 1·6 to 1·9 per cent. less than that obtained by exposure *in vacuo* over phosphoric anhydride for twenty-four hours at 95°. Heating at 110° for two hours is suggested. H. Jephcott (*Analyst*, 1923, **48**, 529) suggests heating at 99° to 100° for two hours in a weighing bottle. The difficulty arises, to a certain extent, from uncertainty as to the hydrated condition of the lactose. Lactose is usually stated to retain its water of crystallisation up to about 130°; this, however, is not the case, and the whole of this water can be driven off by prolonged heating at 100°. Until some standard process is adopted, that of Jephcott would appear to be the most useful.

Fat.—H. Jephcott (*loc. cit.*) prefers the Werner-Schmid to the

Rose-Gottlieb method. Accurate results can, however, be obtained by the latter method, carried out as given under *Condensed Milk* (p. 256), using an equivalent quantity of dried milk.

Solubility.—The following process, due to L. H. Lampitt and E. B. Hughes (*Analyst*, 1924, **49**, 176), is probably the best yet devised. They consider that a milk powder should show a solubility of 99·8 per cent., or thereabouts, of the solids-not-fat.

Method.—(1) To about 38 c.c. of distilled water at 20° C. in a flask of 250 c.c. capacity add 5 grms. of full cream milk powder, or to 45 c.c. of water add 5 grms. of skim milk powder; cork and shake steadily for three minutes. Transfer the whole contents to a tared centrifuge tube, and whirl at about 1,500 revolutions for three minutes. If full cream powder has been taken, now remove any layer of cream on the surface of the milk in the tube, and wipe any cream off the inside of the tube.

(2) Taking care not to disturb the deposit in the tube, pipette off about 5 c.c. of the liquid into a tared nickel dish of weight (w); weigh rapidly; say (a) = weight of dish plus fluid; dry for four or five hours in the steam oven, or about five minutes on the Mojonnier hot plate, and then for fifteen minutes in the Mojonnier vacuum oven; weigh; say (b) = weight of dish plus milk solids.

$(a - b)$ = weight of water lost.
$(b - w)$ = weight of solids.

(3) Now decant as much as possible of the fluid without disturbing the residue, wipe off any cream, etc., on side of tube, and weigh the tube plus residue plus the small quantity of associated fluid; say weight of contents = (c). Then wash out the residue by means of a wash bottle into another tared dish. To hasten drying, alcohol may be used to wash out the deposit. If the amount of insoluble matter is small, it may be dried in the tube instead of being washed out.

Dry in oven as in (2) and weigh.
Say (d) = weight of solids.
Then $(c - d)$ = weight of water lost.

Calculations.—Assume butter fat = Y per cent.; moisture = Z per cent.

Dissolved solids in the fluid contained in $(c) = (c - d) \times \frac{b - w}{a - b} = f$.

Therefore, weight of insoluble solids contained in $(c) = d - f = s$.

Whence, insoluble matter per cent. of powder $= 20s$, and solubility of the powder $= (100 - 20s)$, or

Insoluble matter per cent. of solids-not-fat $= \frac{20s \times 100}{100 - (Y + Z)}$, and

solubility of solids-not-fat $= 100 - \frac{20s \times 100}{100 - (Y + Z)}$.

Dried Milk Calculations.—In England the sale of dried milk is controlled by the Public Health (Dried Milk) Regulations, 1923 and 1927. Under these Regulations Dried Full Cream Milk and Dried Partly Skimmed Milk (of three grades) and Dried Machine-skimmed Milk are all recognised, and standards for the original milk are laid down. Packets have to be labelled with the number of equivalent pints of fresh milk. The calculations required are a little involved, and useful tables have been prepared to assist in these. See E. Hinks, *Analyst*, 1924, **49**, 471; D. Henville, *Ibid.*, 472.

THE EXAMINATION OF BUTTER

The examination of butter falls under two main headings: firstly, that for the admixture of foreign fat, and, secondly, that for proximate analysis and for the detection of dried milk, preservatives and other additions not found in the substance produced by churning milk. For the proximate analysis, the usual methods may be adopted. Thus water may be determined by drying 2 grms. at 100° for two hours, fat and curd by extracting the dried residue with ether, and salt by titrating the residue insoluble in ether with silver nitrate in the usual way. J. M. Jones and T. McLachlan (*Analyst*, 1927, **52**, 383) determine the amount of water by distillation with toluene, which they consider to be better than xylene. G. van B. Gilmour (*Analyst*, 1920, **45**, 173) detects coal tar dyes by melting the fat at a temperature well below 100° (this is important), filtering, and then heating to 180° to 190°: at this latter temperature, butter containing coal-tar dyes remains coloured, whilst others become colourless. L. W. Ferris (*J. Ind. Eng. Chem.*, 1920, **12**, 757; *Analyst*, 1920, **45**, 369) suggests that neutralising agents may be determined in butter and similar products from the ratio of the alkalinity of the salt present to the

inorganic phosphoric acid. D. W. Steuart (*Analyst*, 1928, **53**, 212) determines salt by melting 3 grms. of margarine (or butter) in a 250-c.c. conical flask, adding 10 c.c. of acetone, and titrating with *N* 10 silver nitrate, with chromate indicator as usual. Modifications of Thomson's process for the determination of boric acid have been published (*Analyst*, 1923, **48**, 416; 1929, **54**, 645, 715; 1930, **55**, 23).

The Composition of Butter Fat. The first result of any importance obtained in the quantitative examination of butter fat was that of Duclaux (*Compt. rend.*, 1886, **102**, 1022). This worker, together with Violette (*Compt. rend.*, 1890, **111**, 345; *J. Soc. Chem. Ind.*, 1890, **9**, 1157), Bell, Blyth, Spallanzani, Koefoed, Brown (*J. Amer. Chem. Soc.*, 1899, **21**, 612, 807, 975), Crowther and Hynd (*Biochem. J.*, 1917, **11**, 139; *J. Soc. Chem. Ind.*, 1917, **36**, 1059), has shown that butter fat consists principally of the glycerides of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic and oleic acids. The existence of other acids has been reported, but these, if really existing, must be present in very small quantity.

Further work was published by Holland and his co-workers in 1923 (*J. Agric. Res.*, 1923, **24**, 365; *Analyst*, 1923, **48**, 555), but the composition deduced varies considerably from that reported by Crowther and Hynd. Thus Holland in one case found 2·2 per cent. of butyric acid, whilst Crowther and Hynd found 4 to 4·4 per cent. More recently, T. P. Hilditch and E. E. Jones (*Analyst*, 1929, **54**, 75), have examined New Zealand butter fat by the improved method of alcoholysis due to Armstrong, Allan and Moore (*J. Soc. Chem. Ind.*, 1925, **44**, 63T), and their results must be considered as the most satisfactory up to the present (*cf.* Chapter II., p. 64, *OILS AND FATS*). They found the fatty acids to be combined in the proportion: butyric 3, caproic 2, caprylic 1, capric 2, lauric 4, myristic 11, palmitic 28, stearic 9, oleic 33 to 34, linolic 4 to 5. They also found that about 30 per cent. of the glycerides were fully saturated, whilst there was a tendency for the fully saturated glycerides to contain a smaller proportion of volatile acids than that contained in the partially saturated glycerides. C. A. Mitchell (*Analyst*, 1924, **49**,

515) finds the amount of stearic acid to vary in different samples of butter from 0 to 21 per cent., as determined by the process of Hehner and Mitchell (*Analyst*, 1896, **21**, 316).

The Examination of Butter Fat for Control Purposes.—Many of the standard processes used in analysis may be used with advantage in the case of butter fat. For these, the section on oils and fats should be consulted. The main difference between the composition of butter fat and all other fats likely to be used in its adulteration is the presence of butyric acid, as was first pointed out by Hehner and Angell. Arising directly from their work is the present standard process of Reichert, Polenske, and Kirschner,* which is described in all the reference works on oil and fat examination. This method, whilst quite capable of distinguishing between a pure butter and one containing large quantities of adulterant, is inclined to break down in connection with small additions of foreign fat, other than those of the coconut-oil group. For this reason a large number of new processes and modifications of old ones have been suggested. It must be admitted, however, that few, if any, offer any material advantage over that of Reichert, Polenske and Kirschner, and that there is still a very urgent need for a process that will settle definitely, in difficult cases, whether a given sample of butter fat is or is not adulterated, but from the very nature of things this may be an ideal very difficult of attainment (*cf.* Christian and Hilditch, *Analyst*, 1930, **55**, 75).

As originally discovered by Cribb and Richards (*Analyst*, 1911, **36**, 327), the figures obtained from mixtures of butter with coconut oil are not strictly proportional to the amount of coconut oil present. This subject was continued by Bolton and Revis (*Analyst*, 1911, **36**, 333; 1912, **37**, 183), and more recently by Elsdon and Smith (*Analyst*, 1925, **50**, 53; 1926, **51**, 72; 1927, **52**, 63) have published rather fuller tables. It is very important to remember, however, that all these tables deal with samples of butter and coconut oil having average compositions, and that the indications obtained will be less or more incorrect as the figures for

* It has been pointed out by Elsdon and Stubbs (*Analyst*, 1928, **53**, 212) that the distance between the adaptor and the bulb of the still-head is 78 mm., and not 70 mm., as given by Lewkowitsch and the A.O.A.C. methods.

the component fats of the mixture are near to or far away from the average.

Butters with abnormally low Reichert values are sometimes obtained, particularly from milk towards the end of the lactation period. Such butters are described by H. Lowe (*Analyst*, 1928, **53**, 89) and P. S. Arup (*Analyst*, 1929, **54**, 634). The analyses of two samples of butter, each twenty years old, have been published by F. T. Shutt (*Analyst*, 1925, **50**, 64).

L. V. Cocks and E. Nightingale (*Analyst*, 1928, **53**, 322) draw attention to the necessity for the distillation flask in the Kirschner determination to fit closely to the asbestos ring.

A modification of the Reichert value, originally due to M. Monhaupt (*Chem. Ztg.*, 1909, **33**, 305 ; *Analyst*, 1909, **34**, 212), has been published by G. van B. Gilmour (*Analyst*, 1925, **50**, 272). This is carried out as follows :

Five grms. of fat and 7·5 grms of glycerin are weighed into a small conical flask, and 2 c.c. of a solution of pure potassium hydroxide (1 : 1) are added. The flask is heated over a flame, with constant shaking, until the contents suddenly clear, and after cooling somewhat, about 20 c.c. of distilled water are added. When the soap is dissolved the solution and the rinsings of the vessel are transferred to a 50-c.c. measuring flask, the volume being made up to the mark with distilled water. The soap solution is shaken and then removed to a flask holding about 175 c.c., where the fatty acids are liberated by the addition of 15 c.c. of a solution of sulphuric acid (made either by dissolving 100 grms. of sulphuric acid of sp. gr. 1·825 in water and making up to 1 litre, or by diluting concentrated acid until 11 c.c. just neutralise 2 c.c. of the potassium hydroxide solution). Before the acid is added to the soap solution it is first used to rinse out the measuring flask. The flask containing the liberated acids is now corked and well shaken for about a minute, and the contents filtered through a folded filter paper. When filtering, the aqueous solution is run into the filter paper in such a way that the insoluble acids remain in the flask, which is then corked and shaken vigorously. In this way the acids are made to coalesce and more aqueous solution comes away ; this is added to that already in the filter paper. Fifty c.c. of the filtrate are transferred to a 350-c.c. conical flask, 100 c.c. of distilled water added. After the addition of 0·1 grm. of pumice powder, the flask is connected with a condenser and 100 c.c. distilled, the time of distillation being about twenty minutes. The distillate and washings of the containing vessel are removed to a flask

and, after the addition of a few drops of phenolphthalein solution, the acid in the distillate is titrated with 0·1*N* sodium hydroxide solution. The number of c.c. of alkali required gives the new distillation number of the fat.

C. H. Manley (*Analyst*, 1927, **52**, 67) proposes a sorting test depending upon the saponification of butter fat without distillation. The process is carried out as follows :

Five grms. of the filtered fat are saponified with 20 c.c. of glycerol-soda solution (made by mixing 900 c.c. of pure glycerol with 100 c.c. of a 50 per cent. aqueous solution of sodium hydroxide) and the soap dissolved in 100 c.c. of boiled distilled water. Into the cooled solution 4 drops of 0·5 per cent. methyl orange solution are introduced, and sulphuric acid (25 per cent. by volume) added from a burette until the solution is faintly pink. The total volume of the solution and precipitated fatty acids is taken and 100 c.c. filtered off, nearly neutralised with 10 per cent. sodium hydroxide solution, and the neutralisation completed with 0·1*N* sodium hydroxide solution. In this way the sulphuric acid is neutralised, leaving only the soluble fatty acid, which is then titrated with 0·1*N* sodium hydroxide, after the addition of 0·5 c.c. of 0·5 per cent. phenolphthalein solution. The number of c.c. of 0·1*N* sodium hydroxide solution taken, less the number required for a blank, is represented as the M value.

The method has been criticised by H. S. Shrewsbury (*Analyst*, 1927, **52**, 388), and my experience has shown that it cannot be regarded as a test of precision, as indeed was to be expected from the nature of the colour changes involved.

A table showing the proportion of butter fat in margarine fat, from the result of the Polenske and Kirschner method, has been published by Elsdon and Smith (*Analyst*, 1927, **52**, 65), and the same workers have published a method for the determination of butter fat in margarine fat (*Analyst*, 1927, **52**, 317) based on a process originally devised by van Gilmour (*Analyst*, 1920, **45**, 2). The method is as follows :

Five grms. of butter fat are saponified with glycerin and sodium hydroxide, and the volatile acids are distilled as in the Reichert-Polenske process, with the exception that the distillation is stopped when 100 c.c. have collected, in place of 110 c.c., as is usual. Thirty grms. of pure dry sodium chloride are then added to the distillate, which brings the volume up to 110 c.c. When the whole of the sodium

chloride has dissolved, the solution is allowed to stand for half an hour at 15° C., filtered, and 100 c.c. of the filtrate are collected and titrated with 0·1*N* sodium hydroxide solution to phenolphthalein, the figure obtained being corrected for any acidity found in a blank experiment. This figure, multiplied by $\frac{11}{10}$, gives the salt-soluble volatile acid figure.

The salt-insoluble volatile acid figure is determined in exactly the same way as that for the Polenske process, except that 18 c.c. of sodium chloride solution (30 grms. in 100 c.c. of water) are used in place of 18 c.c. of water to wash the condenser, flask and filter paper.

The distribution of the volatile acid groups among the glycerides of butter fat has been studied by P. Arup (*Analyst*, 1928, **53**, 641), who fractionated butter fat by crystallisation into a lower fraction which was liquid at 10°, a higher fraction which melted at 42° to 46·5°, and four intermediate fractions. The lower fraction was found to have Reichert and Kirschner values about one and one-half times those of the higher fraction, whilst the iodine value was between one and one-half times and twice that of the higher fraction.

J. Kuhlmann and J. Grossfeld (*Z. Unters. Lebensm.*, 1926, **51**, 31; *Analyst*, 1926, **51**, 305) extend the "salting-out" process of van Gilmour (*Analyst*, 1925, **50**, 276) in the following manner:

Five grms. of the fat are saponified with 2 c.c. of potassium hydroxide solution (750 grms. KOH per litre) and 10 c.c. of glycerin, and the soap solution cooled below 100°, and diluted with 100 c.c. of water. The liquid is then cooled to 20°, treated with 50 c.c. of dilute sulphuric acid (25 c.c. H_2SO_4 per litre), 15 grms. of powdered anhydrous sodium sulphate, 10 c.c. of coconut soap solution (pure coconut oil (100 grms.) is saponified by heating it with 100 grms. of glycerin and 40 grms. of potassium hydroxide solution (750 grms. per litre) and the solution, when cold, is made up to a litre), and a pinch (about 0·1 grm.) of purified kieselguhr. The flask is then repeatedly shaken, allowed to stand for ten minutes or longer, its contents filtered through a dry filter, and 125 c.c. of the clear filtrate distilled (after addition of a little pumice-stone) until 110 c.c. of distillate have been obtained in a period of twenty minutes. This distillate is titrated (without filtration) with 0·1*N* sodium hydroxide solution, phenolphthalein being used as indicator. A blank determination, without the fat, but with 10 c.c. of the coconut soap solution, is made, and the difference between the number of c.c. of alkali in the two determinations calculated on 5 grms.

of the fat, and expressed in terms of 0·1*N* solution, is termed the "butyric acid value."

G. van Gilmour (*Analyst*, 1921, **46**, 183; 1925, **50**, 119) has determined the melting points of the insoluble acids obtained in the distillation process, and draws certain conclusions from them.

F. Bamford (*Analyst*, 1924, **49**, 226) examined the Denigès test for butyric acid with the idea of using it as a qualitative test for butter fat in margarine fat. He found, however, that the test is also given by caproic and by caprylic acids, normal constituents of margarine fat, so that the test is of no value from this point of view.

The determination of butter fat by extracting the fatty acids with xylene has been suggested by A. van Raalte (*Chem. Weekbl.*, 1926, **23**, 222; *Brit. Chem. Abst.*, 1926, **1***B*, 563):

Five grms. of the fat are saponified, the free acids distilled in steam, 110 c.c. of distillate collected and filtered. One hundred c.c. of the filtrate are shaken with 10 c.c. of water and 22 c.c. of xylene, the aqueous layer is filtered, and 100 c.c. are titrated with 0·05*N* sodium hydroxide. The volume required, in c.c. − 1·21 ; 2, is the xylene number. The percentage of butter in a fat is calculated from the formula (xylene number − 0·63) ÷ 0·23.

A more recent method for the examination of butter fat has been suggested by H. Atkinson (*Analyst*, 1928, **53**, 520) and applied by him particularly to Egyptian butter fat. This is carried out by distilling the acids from 1 grm. of saponified fat with 210 c.c. of water, determining the acids insoluble in 62 per cent. aqueous alcohol, the oleic acid, and the residual acids. The new value is obtained by subtracting the oleic acid and the acids insoluble in 62 per cent. alcohol from the non-volatile acids. This for butter is 36, beef fat 14, coconut oil 146.

Separation of Unsaponifiable Matter. The detection of coconut oil in butter fat by means of the unsaponifiable matter is carried out by C. F. Muttelet as follows (*Compt. rend.*, 1922, **174**, 220; *Analyst*, 1922, **47**, 259):

Fifty grms. of the fatty acids and unsaponifiable matter are treated with 20 c.c. of a 1 per cent. solution of digitonin in 95 per cent. alcohol,

frequently stirred, and, after standing thirty to forty-five minutes, heated and filtered. The addition of 1 c.c. of water at an early stage promotes separation of the digitonides. The precipitate is washed with hot chloroform and cold ether and then dried. It is then boiled for five minutes with 2 to 4 c.c. of acetic anhydride, and the sterol acetate precipitated by the addition of 5 volumes of 50 per cent. alcohol. The precipitate is filtered off, dissolved in cold ether, evaporated to dryness and recrystallised from 1 to 2 c.c. of absolute alcohol. Cholesteryl acetate, thus separated, melts at 113·6° to 114·2°, whilst phytosteryl acetate from coconut oil melts at 125°. Butter with 5 per cent. of coconut oil gives crystals melting at 115·5°.

A. More (*Analyst*, 1929, **54**, 735) recommends Dr. van Sillevoldt's method, which is as follows:

Saponify, with reflux condenser, 15 grms. of filtered fat with 9·5 ml. of potassium hydroxide solution (1,000 grms. of KOH in 1,400 ml. of water) and 20 ml. of alcohol (96 per cent.) in a 300-ml. conical flask. Shake while warm until the fat is dissolved, and heat further for half an hour.

Cool, add 60 ml. of water and 180 ml. of alcohol (96 per cent.), mix, and add 10 to 20 ml. of digitonin solution (1 per cent. of Merck's digitonin in 96 per cent. alcohol). Allow the mixture to stand for twenty-four hours in a cool place and filter on a Buchner funnel with a closely fitting paper. Wash with a small amount of alcohol to remove soap. The digitonin-sterol compound flakes off on drying. Weigh the steride and acetylate it with ten times its weight of acetic anhydride, and proceed with the crystallisation from alcohol (about 95 per cent.) as in the Bömer method.

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See also Chapter II.

CHAPTER VIII

PAPER

By William Dickson, F.I.C.

Determination of Nature and Proportions of Fibres: Composition of Stains for Differentiation—Method of Making an Analysis—Use of Polarised Light—Chemical Methods of Determining Mechanical Wood Pulp. *General Analytical Examination:* Moisture—Proteinaceous Materials—Starch—Colouring Matters—Miscellaneous Materials. *Physical Tests:* Weight—Thickness—Bulk—Breaking Strain, etc.—Air Porosity—Internal Sizing—Filter Paper—Blotting Paper.

Introduction.—While the various commercial fibres of which it is composed affect the quality and durability of paper, other factors, as, for example, the amount of inorganic fillers incorporated, or the type and quantity of sizing material used, have their effects also. In every case the analyst can render help. At the same time, when the utmost has been done from what may be termed the purely analytical side, there remain differences of quality and behaviour which can be neither readily detected nor controlled by analysis, such as, for instance, varying degrees of "hydration" produced during the beating operation. For this reason paper technologists have recourse to a number of physical tests, such as breaking strain, stretch, resistance to folding, etc., to characterise and control their product.

In view of these considerations, the subject of paper examination may be conveniently divided into three parts:

(1) Determination of the nature and proportion of fibres present. (2) General analytical examination. (3) Physical tests.

DETERMINATION OF THE NATURE AND PROPORTIONS OF THE FIBRES PRESENT

In paper-maker's parlance, this is called determining the "furnish." The method of carrying it out entails a microscopic

examination of the pulp after the paper has been disintegrated. If the chemist is an expert he may estimate with fair accuracy the proportions of a mixture by the rapid examination of a few slides. For the ordinary chemist, however, the process involves a long and laborious series of counts.

Before consideration is given to detailed methods some general observations will be helpful.

By far the most commonly used stain for distinguishing between the various fibres in paper is Hertzberg's, which consists of a potassium iodide solution of iodine and a solution of zinc chloride. The rationale of the action of this stain upon cellulose fibres, according to Cross and Bevan (*Paper Making*, p. 93), is that the zinc chloride exerts a chemical hydrating action on the cellulose, and that the transition from red to pure blue is the effect of this. The resistant celluloses, cotton, flax, hemp, etc., are less sensitive than those prepared from igno-cellulose materials by severe chemical treatments. It is not surprising, therefore, that the distinguishing tints produced by this stain and others of similar nature are not very definite. In using them it should be realised that their main function is to bring out the characteristic appearance of the fibres. The colours are aids to diagnosis rather than definite tests for individual fibres, and the analyst must trust very largely to his knowledge of the morphological appearance of fibres in making his counts. Again, the beating of the paper pulp breaks up the fibres to a varying extent and tends to alter their appearance, which naturally adds to the difficulties.

From these considerations it will be evident that practice is necessary to ensure success. The analyst should first work on known fibres beaten to correspond more or less with the condition in which they occur in a paper. He should check himself also at every turn by making up mixtures of known proportions and estimating them as nearly as he can. The mixtures should preferably be made up by an independent operator, and the proportions disclosed only after the analysis is completed.

For literature on the subject see references (1), (2) and (3) in the Bibliography at the end of this Chapter.

Composition of the Various Stains used in the Differentiation of Paper Fibres

The details of the following stains and of the methods of using them are largely taken from *Paper Testing Methods*. As these are official for the American pulp and paper industry, they may commend themselves to the general chemical practitioner.

(1) **Hertzberg's Stain.**—This comprises: (*a*) An aqueous solution of pure zinc chloride saturated at 70° F.

(*b*) A solution of 0·25 grm. of resublimed iodine and 5·25 grms. of pure potassium iodide in 12·5 c.c. of distilled water.

A mixture of 25 c.c. of (*a*) with an equal quantity of (*b*) is allowed to stand till clear. The clear liquid is decanted into an amber-coloured glass bottle and a crystal of iodine added.

(2) **Sutermeister's Stain.**—(*a*) 1·3 grms. of iodine and 1·8 grms. of potassium iodide in 100 c.c. of water. (*b*) A clear saturated solution of calcium chloride.

(3) **Iodine in Potassium Iodide Solution.**—This contains 2 grms. of iodine and 10 grms. of potassium iodide dissolved in 40 c.c. of water.

(4) **Lofton Merritt's Stain.**—(*a*) Malachite green, 2 grms. in 100 c.c. of water. (*b*) Basic fuchsine, 1 grm. in 100 c.c. of water.

For use, 1 part of (*a*) and 2 parts of (*b*) are taken.

(5) **Bright Stain.**—(*a*) 2·7 grms. of ferric chloride in 100 c.c. of distilled water. (*b*) 3·29 grms. of potassium ferricyanide in 100 c.c. of distilled water. (*c*) Three grms. of crude substantive dye (benzopurpurine 4BS, British Dyestuffs Corporation) in 250 c.c. of distilled water.

METHOD OF MAKING AN ANALYSIS

A sample, of at least 1 square inch, cut from different portions of the sample submitted, is taken, covered with 0·5 per cent. caustic soda solution and heated to boiling. The mixture is placed on a 200-mesh sieve, and the paper thoroughly washed with water. The moist pieces of paper are rolled together in the fingers and worked sufficiently to loosen the fibres, after which the mass is transferred to a test-tube and shaken with water until the fibres are completely separated. A portion of the mixture is poured into a second test-tube and diluted to a fibre

concentration of about 0·1 per cent. A sample of fibre is transferred to a microscope slide by means of a dropper consisting of a glass tube, 6 inches long and $\frac{1}{4}$ inch internal diameter, fitted at one end with a rubber bulb. The procedure is to mix the sample solution, insert the dropper 2 inches below the surface of the liquid, expel two bubbles of air from the dropper, fill it to about $\frac{1}{2}$ inch, and transfer the contents to the slide, making 4 drops empty the dropper completely. This procedure is repeated until the slide is completely covered with drops. It is then placed in an air oven to dry, after which it is stained by the method best suited to the solution to be used. A second microscope slide or cover-glass is placed on top.

(1) **Hertzberg's Test.**—The fibres are thoroughly moistened by the solution, the excess being removed with filter paper before applying the cover-glass.

The stain must be standardised on a known mixture. For example, a mixture of about equal parts of bleached soda pulp, bleached sulphite pulp and rag filter paper may be taken, and a slide made up and stained. If the stain is correct, the soda pulp should show a dark blue colour, the sulphite a light blue, and the rag fibres a red or wine-red. If the blue colour is violet rather than blue, too much iodine is present, and more water or zinc chloride solution should be added. Zinc chloride produces the blue colour, iodine the red, while water serves to weaken the predominating colour.

Stains standardised on different pulps are sometimes required. For example, if mechanical wood is present, the stain should be adjusted to give a bright lemon-yellow on this ingredient. The unbleached sulphite will then be coloured greenish-blue. Differentiation between bleached sulphite and soda pulp will be very difficult. For this a separate slide should be made with the stain standardised according to the example first given.

(2) **Sutermeister's Test.**—The fibres are moistened with water. Solution (*a*) is applied and allowed to act for one minute. The excess is removed, solution (*b*) added and the slide covered.

(3) **Iodine Potassium Iodide Solution.**—The fibres are moistened with the solution, the excess removed, and the cover-glass put on.

(4) **Lofton Merritt's Test.**—This solution distinguishes between unbleached sulphate and unbleached sulphite fibres, the former

giving a blue or blue-green colour, the latter a purple or lavender. If any purple fibres appear in unbleached sulphate, too much fuchsine is present and more malachite green solution should be added; if unbleached sulphite develops a green or blue colour, more fuchsine is required. The compound stain is added to the fibres and allowed to act for two minutes. The excess is removed by filter paper, and a few drops of 0·1 per cent. hydrochloric acid added. This is allowed to act for thirty seconds, the excess removed, and distilled water added. Again the excess is removed, and the cover glass placed in position.

(5) **Bright Stain.**—This is used for differentiating bleached and unbleached fibres; the former develop a red, the latter a blue coloration. Ten c.c. each of (*a*) and (*b*) are placed in a tall, narrow beaker, and an equivalent amount of (*c*) in another beaker. The beakers are placed in a water-bath maintained at 20° C. (± 1°). The slide is dipped in distilled water so that it is uniformly moistened, then transferred to (*a*) + (*b*), support being given by means of a clamp. It is allowed to stand for twenty minutes, after which it is washed by being dipped six times into distilled water. The water is changed, and the dipping repeated six times. The slide is dried and the same process used in staining it with (*c*) solution. A drop of Canada balsam is applied to the slide, and a cover-glass placed on top.

Stains (1), (2) and (3) are intended for general use. The table on p. 277 summarises the colours obtained with the various fibres.

A set of eight coloured plates serving as standards for the identification of pulp fibres is published in Technological Paper No. 250, *Pulp and Paper Fibre Composition Standards*, by the Bureau of Standards, U.S. Department of Commerce. These should prove useful for reference.

It can be plainly seen from the table that the colours developed are not very definite. Knowledge of the morphological appearance of the various fibres is necessary, a knowledge best obtained by examination of known fibres, and mixtures of them, prepared and stained by the analyst himself. Considerable help may also be obtained from published photo-micrographs appearing in the

DIFFERENTIATION OF FIBRES BY STAINING TESTS.

	Fibre.	Colour after Staining.		Fibre.	Colour after Staining.
		Iodine Potassium Iodide Solution.	Hertzberg.		Suberinester
Group 1 — Lignified fibres	Mechanical wood. Raw jute not quite opened out.	Part light yellow, part brownish yellow, according to thickness of layers and kind of lignified fibre.	Citron yellow to dark yellow.	Mechanical wood.	Yellow.
	Straw pulp.	Part brownish-yellow, part yellow, part grey.	Part yellow, part blue, part blue-violet.	Jute, manila hemp, sulphite cellulose unbleached and only partly lignified.	Greenish.
Group 2. — Cellulose fibres.	Wood cellulose and adansonia.	Grey to brown.	Blue to red-violet.	Completely opened out or bleached sulphite cellulose.	Bluish or reddish-violet.
	Straw and jute cellulose.	Grey.	Blue to blue-violet.		
	Esparto.	Part grey, part brown.	Part blue, part wine-red.	Bleached soda pulp from deciduous wood.	Dark blue.
	Manila hemp.	Part grey, part brown, part brownish-yellow.	Blue-violet, red-violet, dirty yellow, greenish-yellow.		
Group 3. — Rag fibres	Linen, hemp, cotton, ramie.	Light to dark brown, thin laminæ, almost colourless.	Weak to strong wine-red.	Linen, hemp, cotton, ramie.	Red or brownish red.

books of reference already quoted. There is a very exhaustive table giving fibre characteristics in *Paper Testing Methods*, pp. 26, 27.

Counting.—As regards the method of counting, observations should be made on different fields in a straight line, twice lengthways and four times crosswise. A microscope with a magnification of 60 diameters should be used, while a higher magnification (100 diameters) will be useful for a closer inspection of the structure of individual fibres. The number of diameters of each kind of fibre in, at least, twenty-five different fields should be counted. The diameter of the field, as seen through the microscope, is the unit of measurement. The numbers found should be expressed as a percentage of the total fibre composition. For example, suppose the total diameters of wood fibres to be 92, and those of hemp to be 18 (other fibres being absent), the percentage of hemp will be :

$$\frac{18 \times 100}{(92 + 18)} = 16 \text{ per cent.}$$

The "dot" method of counting, given as tentative in the American official methods, has something to commend it, as it tends to eliminate the personal equation (*Paper Trade J.*, 1929, **22**, 44). To carry it out, cement a crossline disc to the eyepiece diaphragm of the microscope. (A satisfactory disc may be produced by cementing two silk fibres at right angles across a cover-glass with paraffin wax or other adhesive.) Start at one end of the slide about one-quarter of the way down from the top, and move the slide throughout its entire length by means of the mechanical stage. In moving count each fibre, or part of a fibre, which passes directly under the point or dot formed by the intersecting lines of the disc. Some long fibres may pass twice under the dot, but they should be counted each time. If aggregations of fibres are encountered, such as occur in mechanical wood, the number of single fibres is estimated and counted as if they were completely separate. Select two other paths across the slide, and two or three up and down, taking care to cover the whole area in a systematic manner. Add the counts together and calculate the composition as before.

PLATE I.

FIG. A.—Hemp (ordinary light) × 300 diams.

FIG. B.—Hemp (polarised light) × 300 diams.

FIG. C. Long wood cell easily mistaken for hemp (ordinary light) × 67 diams.

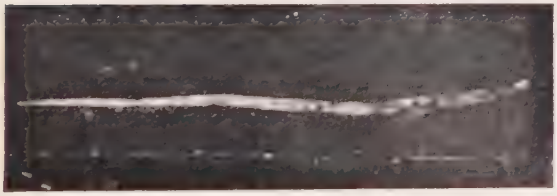

FIG. D.—Long wood cell (polarised light) × 67 diams.

(To face p. 278.

FIG. A.—Hemp (ordinary light) × 67 diams.

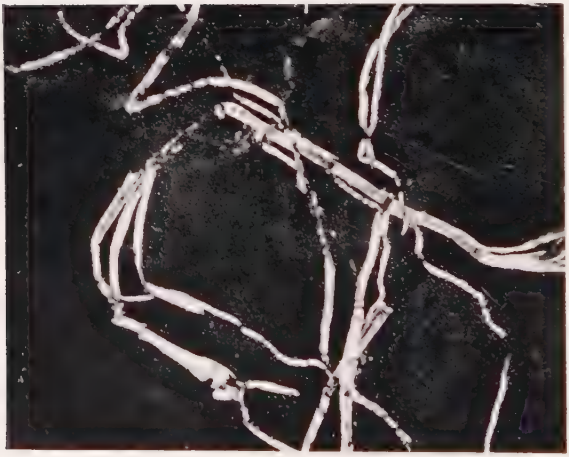

FIG. B.—Hemp (polarised light) × 67 diams.

Use of Polarised Light.—The above well-known methods are difficult and inaccurate, figures correct to the nearest 5 per cent. being considered good : anything tending to greater ease, certainty, and therefore accuracy, is of advantage to the paper chemist. Polarised light has been found useful in differentiating such fibres as hemp and wood in paper pulp (Dickson, *Analyst*, 1923, **48**, 373). Its use, in conjunction with a special ammoniacal silver nitrate stain, has led to a clear differentiation between cotton and linen (*Analyst*, 1925, **50**, 317), while it has been employed with textile fibres also (*Indust. Chemist*, 1929, 147).

Examination by polarised light can quite well be carried out with specimens stained by the American official methods, and can, with advantage, be looked upon as supplementary thereto. All that is necessary is to turn the polariser and note the change in appearance of any doubtful fibre. This does not, of course, apply to the differentiation between linen and cotton obtained by the special ammoniacal silver nitrate stain. This must be dealt with as a special stain for a special purpose. Plates I. to VI. show the appearance of certain fibres under ordinary and under polarised light.

Certain points arising out of my work on this method may be of interest.

(1) It is considered preferable to work at a lower fibre concentration than 0·1 per cent., as recommended by the American official method previously cited. The concentration of 0·02 to 0·03 per cent., suggested by Spence and Krauss (*World's Paper and Trade Rev.*, December 18th, 1917), is better.

(2) The dropper method of transferring the pulp to the slide, though expeditious, tends to put too many fibres in one place. The subsequent counting is difficult and liable to inaccuracy.

In view of the above considerations, the following procedure for disintegrating paper and making slides is recommended :

A quantity of 0·5 grm. of paper, taken from various parts of the sample, is weighed out and boiled with 100 c.c. of 10 per cent. sodium hydroxide solution to remove the sizing materials. Parchment paper is disintegrated by heating with dilute (1 : 1) sulphuric acid solution at 50° to 60° instead of sodium hydroxide. Papers coated with gelatin require

to be first treated with warm water to swell the gelatin, then boiled with water to remove it, and finally boiled with sodium hydroxide in the usual way. The paper is pulped in a mortar until most of its structure disappears. The pulp is transferred to a cylindrical glass vessel, A, 5 inches high by 3½ inches in diameter, and thoroughly pulped by means of an egg switch. When the pulp is quite disintegrated, it is placed in a Winchester quart bottle, which is filled up with water and well shaken. This mixture is of the correct fibre concentration (0·02 per cent.).

A sample is poured off into another glass cylinder, B, about 6 inches high by 2 inches in diameter, then filtered off on a small piece of 200-mesh gauze and thoroughly washed. The pulp is replaced in cylinder B and made up to the same height as previously occupied, to keep the concentration right. The mixture is now returned to cylinder A and again pulped with the egg switch to loosen any clots formed on the filter. It is stirred with a microscope teasing needle, so as to make it rotate quickly. The needle is then held stationary for a few minutes near the side of the glass, when the pulp will attach itself to the needle and a good average sample be obtained by simply withdrawing the needle. Three slides are prepared, one needle sample to each. This is very carefully teased out by means of the needle and a small scalpel, so that individual fibres are seen under the microscope. Very considerable practice is required to achieve this result, but time spent on it is repaid by the greater ease experienced in the counting operation. Excess water is removed from the slides, and they are dried in an air oven.

The general chemist, who may not possess a model hollander and other special apparatus for making paper pulp, may use the following method for preparing authentic samples of pulp for making mixtures, to standardise stains, check counts, etc. :

Fifty grms. of the material, *e.g.*, hemp rope, are boiled with 10 per cent. sodium hydroxide solution for several hours. The liquid is removed on a Büchner funnel, and the residue pulped in a hand mortar. It is then boiled with an acid sodium sulphite solution, filtered, and pulped. Afterwards it is warmed with sodium hypochlorite solution, acidified and the mass kept warm for a short time, when it should be bleached to a good white colour and resemble paper-maker's " half stuff " in fineness. In making mixtures dry pulp is weighed out in the requisite proportions, mixed with water and beaten with an egg switch, used as previously described for disintegrating paper. The beating must be continued until the material corresponds in form with the paper pulp with which it is to be compared.

FIG. A.—80 per cent. hemp, 20 per cent. wood (ordinary light) × 67 diams.

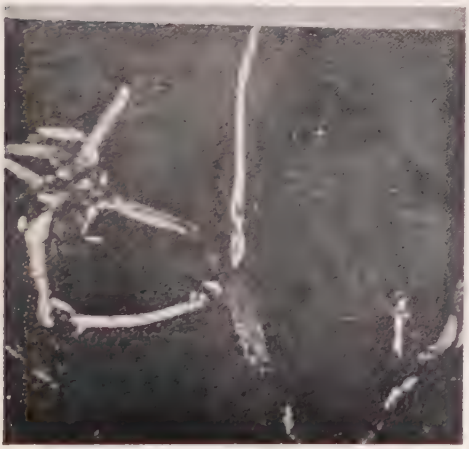

FIG. B.—80 per cent. hemp, 20 per cent. wood (polarised light) × 67 diams.

[*To face p.* 280.

FIG. A.—Wood (under ordinary light) × 67 diams

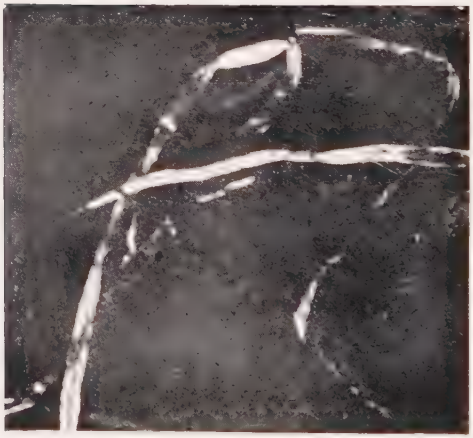

FIG. B. – Wood (under polarised light) × 67 diams.

Differentiation of Cotton and Linen.—Details of the method of distinguishing between cotton and linen are as follows :

A stock solution of 5 grms. of silver nitrate and 100 c.c. distilled water is made up, and 10 c.c. of this solution are placed in a test-tube, and immediately before use 20 per cent. ammonium hydroxide solution is added until the silver hydroxide first precipitated is just dissolved. The pulp, prepared as in the previous method up to and including the filtration and washing on the 200-mesh gauze (p. 280), is now added, and the whole heated in a boiling water bath for thirty minutes. It is then filtered off through a small tough filter paper and well washed with distilled water. Ten c.c. alcohol are added and the pulp gently stirred, care being taken not to injure the filter paper. The filter is allowed to drain, then a further 10 c.c. alcohol permitted to drain through. The filter is placed in a water oven to dry, the pulp separated from it and thoroughly teased out by means of a pair of fine forceps and a small scalpel. Twenty c.c. of a solution containing 2 c.c. of nitric acid (sp. gr. 1·42) in 100 c.c. of water) are placed in a crystallising dish and heated to 70° to 80° on a water bath. The teased pulp is added and stirred frequently for five minutes. The mixture is finally diluted to the concentration originally started with, and slides made as described (p. 280). An examination under ordinary and polarised light enables the fibres to be distinguished. Linen appears black in ordinary light, whilst cotton is almost invisible ; linen is almost invisible in polarised light, whilst cotton shows up very distinctly (see Plates IV. and V).

CHEMICAL METHODS OF DETERMINING MECHANICAL WOOD PULP

Mechanical wood pulp can be determined with fair accuracy by the use of the microscopic methods already given, provided the chemist possesses the necessary experience and is prepared to devote the necessary time and care to the process. There are other methods, however, depending on chemical reactions, as distinguished from microscopical examination, which are quicker and, at the same time, perhaps more convenient for one not specially expert with the microscope.

The best known of these utilise the phloroglucinol reaction for lignified fibre. Two applications of the method are available :

(1) Colorimetric, for papers with low percentages of mechanical wood.

(2) Volumetric, for papers with high percentages of mechanical wood.

Details of the methods may be obtained from Cross and Bevan's *Paper Making*, 5th Ed., p. 416.

Methods based upon the lignin content have been suggested. This can be determined in a number of ways, *e.g.*, by the Willstätter method (*Papierprüfung*, p. 145), which has recently been improved by Schwaller and Ekenstam (*Cellulosechem.*, 1927, **8**, 13; 1927, 183), who have shown that it is preferable to treat the wood first with 25 per cent. hydrochloric acid, and later to raise the concentration to 42 per cent. This procedure is intended to avoid contamination with unresolved cellulose due to the rapid swelling action of concentrated acid. Such methods, however, are of little use unless samples of the original pulp are available, since different pulps show widely divergent figures for lignin (*Papier-J.*, 1926, **10**, 121; *B*, 1927, 8).

DETERMINATION OF LENGTH OF FIBRE

This is an important matter, because, as is well known, other things being equal, long fibres give a stronger paper than do short fibres.

To carry out a test, slides are made up as for the determination of fibres. Individual fibres are measured on three slides, care being taken to select a sufficient number of fields equally distributed over the slides. An eye-piece scale, or, better, an eye-piece micrometer, as well as a stage millimetre scale, together with a microscope giving a magnification of 40 diameters, are required. The value of the divisions of the eye-piece scale or the micrometer eye-piece must be determined in relation to the divisions on the stage millimetre scale. The microscope is focussed on the millimetre scale, the eye-piece turned so that its lines lie along those of the millimetre scale. The divisions of the scale, equal to 1 mm. on the millimetre scale, are counted, the process being repeated once or twice, and the average taken. The slide to be examined is then placed on the stage, and the fibres measured in terms of the eye-piece scale. The measurements are converted into millimetres

and fractions of millimetres, taking the previously determined value for 1 mm. The eye-piece, objective, and length of tube must be kept constant throughout a determination, since any change will affect the value of 1 mm. in terms of the eye-piece scale.

Type of Beating.—The control of the beating of paper pulp is a matter of controversy. This is not the place to go into the methods either of carrying out or of controlling beating, but it may be mentioned that the aspect of the beaten pulp under the microscope, the length of fibres, the appearance of their ends—whether sharply cut or frayed—the number of fibrillæ or broken-away pieces, etc., all afford useful clues to the type of beating. Photomicrographs of known beatings of known fibres or mixtures of fibres are useful for comparison, but it will be realised that much experience on the part of the chemist is required for satisfactory diagnosis.

GENERAL ANALYTICAL EXAMINATION

In dealing with this section, it has been considered best to collect together those methods which have proved satisfactory, and to give them in detail without entering into a discussion (which to be adequate must needs be lengthy) of their underlying principles. These methods have, in most instances, the sanction of the Technical Association of the Pulp and Paper Industry of America.

Moisture

A sample, not less than 2 grms., is taken from different parts of the larger test sample, or different reams in the case of a consignment, placed in a weighing bottle, and weighed to the nearest mgrm. If the paper, as is usual in the case of a consignment, is not in equilibrium with the atmosphere, the manipulation should be carried out quickly, for changes in moisture content take place rapidly when the sample is withdrawn from the mass. The stopper of the weighing bottle is removed, and the whole placed in a water oven for an hour. The bottle is then stoppered, placed in a desiccator till cool, and again weighed, drying and weighing being repeated till the sample remains constant in weight. The moisture is calculated to percentage on the sample as received, or the result may be calculated on the dry paper, so long as it is stated in the report which method has been employed.

The determination of moisture (*cf.* Stevens, *The Paper Mill Chemist*, p. 213) in a consignment of wood pulp, however, is a special case.

(*a*) *Sampling.* Not less than 2 per cent., or more than 4 per cent., of the total number of bales should be sampled. Three to five sheets from each bale should be taken, and four wedges cut from each sheet, the apex of each wedge being in the centre of the sheet, and the base on each side. Suppose three sheets are taken from a bale, one 1½ inches from the top, one 1½ inches from the bottom, and one from the middle. If the bale measure 33 inches in depth, the sheets from top and bottom will represent 6 inches of the bale, and the one from the middle will represent 27 inches. Nine times as much sample should, therefore, be taken from the middle sheet as from the other two. If the base of the wedges from the two outer sheets is ½ inch, that of the wedges from the middle sheet should be nine times as great, or 4½ inches. The samples should be placed at once in air-tight tins.

(*b*) *Determination of Moisture.* The weight of pulp should be determined from the weight of (pulp + tin) − weight of tin. The pulp is dried to constant weight in a water oven. When nearly dry weighings should be made every half-hour. The pulp may be weighed quickly while hot without appreciable error, a balance sensitive to 0·01 grm. being used. The result should be calculated as percentage of bone-dry pulp as well as percentage of pulp containing 10 per cent. of moisture, or, in the case of moist pulp, 55 per cent., as these are the bases for usual contracts. If the weight of the total consignment is known, the correct weight to be involved can be deduced.

Ash. A sample, consisting of at least 1 grm., is placed in a platinum, alundum or porcelain crucible with a tightly fitting lid and weighed. The lid of the crucible is raised and the paper gradually burned away, care being taken that no small particles are lost during the process. When the ash is burned white, the crucible, with lid on, is cooled in a desiccator and weighed. A second ignition is given to ensure that constant weight is attained. The percentage of ash is reported on the sample as received. The natural ash of most paper-making fibres is about 1·5 per cent., any material increase on this figure indicating the presence of inorganic filling in the paper.

Inorganic Filling. A qualitative examination of the ash, of which about 0·2 grm. is required, is made by the usual methods of inorganic analysis.

The ash is first extracted with hydrochloric acid, and the soluble part examined separately. The residue is then fused with fusion mixture, and the fused mass dissolved and examined. The table on p. 285 will enable a diagnosis of the constituents to be made.

Part soluble in acid.		Part soluble in acid after fusion.	
Calcium and sulphate in quantity.	Crown filler. $CaSO_4,2H_2O$.	Barium and sulphate.	Blanc fixe or barytes.
Alkaline ash with calcium, but no sulphate.	Whiting or chalk, $CaCO_3$.	Aluminium and silica in quantity.	China clay.
Ash effervescent and showing barium in quantity.	Barium carbonate.	Magnesium, silica in quantity.	Talc or asbestine.
Calcium, aluminium and sulphate.	Satin white.	—	—

Occasionally papers sized with sodium silicate are met with. Since some of the sodium silicate becomes converted into aluminium silicate through treatment with alum in the beater, the presence of aluminium silicate in the ash may lead to the erroneous conclusion that the paper is loaded with china clay.

A microscopic examination of the ash will always assist in determining the kind or kinds of mineral filling present.

If it is necessary to determine the quantity of the inorganic filling present, this can be done by the customary methods of inorganic quantitative analysis.

Mineral Coating. The weight of a piece of paper, 2 inches by 5 inches, in a normal atmosphere (see p. 294) is obtained. The paper is placed on the bottom of a flat tray, and a warm 5 per cent. solution of hydrochloric acid poured over it. This is allowed to act for a few moments, then poured off. A warm 5 per cent. solution of ammonium hydroxide is next poured on, the paper being moved round so that all parts of it come into contact with the solution. It is then placed on a glass plate, and the coating brushed off with a camel-hair brush, care being taken not to dislodge any fibre. Next, the paper is washed by means of a wash bottle, the glass plate being held at an angle and the paper kept in position by pressure at one corner. It is dried and the weight in a normal atmosphere obtained. Care should be taken that true equilibrium is reached; the sample should be replaced for a few hours in the normal atmosphere, then re-weighed. The difference between this and the original weighing gives the amount of coating present. Two

determinations, at least, should be made to see that they are reasonably concordant.

If it is desired to determine the nature of the mineral constituents of the coating, a sample of not less than 0·2 grm. of the coating, obtained by the above-described method of separation, should be analysed by methods similar to those given under "Inorganic Filling," p. 284.

Casein.—Casein and glue are frequently used as coatings or surface sizing materials. The presence of casein may be established by the Millon reaction :

Five grms. of the sample are boiled for several minutes with 10 c.c. of 1 per cent. sodium hydroxide solution (caustic soda is required to dissolve casein which has been hardened by formaldehyde or other reagents). The aqueous extract is filtered off, cooled to room temperature and exactly neutralised to phenolphthalein by means of nitric acid. A few c.c. of Millon's reagent are added.* The presence of casein is indicated by the development of a red coloration on heating, which is due to the presence of tyrosine, of which casein contains about 5 per cent. It is only rarely present in animal glue or gelatin, and then only in doubtful traces.

Nitrogenous Proteinaceous Materials.—*Qualitative Tests.*—The presence of both glue and casein is shown by the development of a white precipitate when ammonium molybdate is added to the sodium hydroxide extract of paper after neutralisation to phenolphthalein with nitric acid. The test is very delicate, and if there is only a slight precipitate, no appreciable amount of proteinaceous materials can be present.

The ammonium molybdate solution is prepared by dissolving 3 grms. of pure ammonium molybdate in 250 c.c. of distilled water, and adding 25 c.c. of pure nitric acid (sp. gr. 1·2). The reagent is not permanent and should be freshly made up at frequent intervals.

The "chloroamine" test (*Paper Making*, p. 420) for proteinaceous matter may also be used. It is convenient for the detection of one-side treatment, spots, stains, etc.

Quantitative Determination. The well-known Kjeldahl-Gunning

* Millon's reagent is prepared as follows: Dissolve 20 grms. of pure mercury in 40 grms. of concentrated nitric acid (sp. gr. 1·42). Dilute the solution to 180 c.c. with distilled water.

method is employed. From 3 to 5 grms. of the sample are required, and a 500-c.c. Kjeldahl flask is best. The percentage of nitrogen found, after allowing for any blank due to the reagents used, is multiplied by 7·69 (Sutermeister, p. 400) or 6·3 to give the percentage of commercial casein or glue respectively. These factors allow for the ash and moisture normally present in the commercial articles. Since the nitrogen content of the materials varies somewhat, samples of the actual materials used in the sizing should be procured, if possible, and the calculation based on their nitrogen content, as determined by the Kjeldahl-Gunning method.

A rough determination of casein or glue in the absence of any other soluble materials in the paper may be made by taking the weight in a normal atmosphere of a specimen of the paper, say, 2 inches by 5 inches, boiling it out with 1 per cent. sodium hydroxide solution, as in the qualitative test for casein previously given, washing thoroughly with water, drying, and again ascertaining its weight in a normal atmosphere.

Starch in Paper. Starch also is used as sizing material for paper. Its presence may be detected by boiling out 0·5 grm. of the paper with water, filtering off the extract, cooling it and adding 1 drop of *N*/10 iodine solution. A distinct blue coloration establishes the presence of starch. A faint violet colour, however, is inconclusive, since non-starch constituents of paper sometimes give this reaction.

Quantitative Determination of Starch in Paper.—Five grms. of paper, cut up into small pieces about ¼ inch square, are boiled under a reflux condenser with 200 c.c. of water and 5 c.c. of glacial acetic acid for one and half hours. The contents of the flask are poured on to a Buchner funnel, and the pulp washed with 50 c.c. of hot water, with the aid of suction. To the filtrate are added 15 c.c. of 37 per cent. hydrochloric acid, and the solution is boiled for thirty minutes, the volume being allowed to decrease to about 200 c.c. The hot solution is neutralised by adding solid sodium carbonate until effervescence ceases, cooled, and made up to a definite volume in a volumetric flask. The solution is titrated into a measured quantity of Fehling's solution, diluted to 25 c.c. After each addition the solution is boiled for one minute. The end-point is determined by adding a drop of the mixture to a mixture of one spot of 10 per cent. potassium ferrocyanide solution and one spot of 20 per cent. acetic acid on a white porcelain spotting plate. The end-point is reached when no immediate colour is produced on mixing the solutions.

The Fehling's solution consists of: (*a*) 69·3 grms. of crystallised copper sulphate made up to 1,000 c.c. with distilled water.

(*b*) Three hundred and forty-six grms. of Rochelle salt and 120 grms. of sodium hydroxide made up to 1,000 c.c. with distilled water.

The solutions are allowed to stand for two days, then filtered. For use, equal quantities of (*a*) and (*b*) are taken. One c.c. of the solution is equivalent to 0·005 grm. of starch.

If the starch used in the paper is available, the factor should be determined for the sample in question, since different grades vary in their copper reduction value.

To standardise the solution, weigh out 0·05 grm. of starch, dried at 100° to 105°, and boil with 190 c.c. of a 4 per cent. hydrochloric acid solution for thirty minutes. Neutralise with solid sodium carbonate, cool, adjust to a definite volume, and titrate into a definite volume of Fehling's solution, as described above.

Resin.—Resin size (sodium resinate) is the most generally used sizing material for paper. Its presence may be definitely concluded if both the following reactions give positive results :

(1) *Liebermann-Storch Reaction.* One grm. of paper, cut up into small pieces, is placed in a dry test-tube, and 5 c.c. of pure acetic anhydride added. The liquid is boiled down to about 1 c.c., and the residue poured into a clean, dry porcelain crucible. The mixture is cooled to room temperature, and any waxy particles which separate are filtered off. One drop of concentrated sulphuric acid is added carefully down the side of the crucible. A fugitive rose-violet coloration, developed where the acid meets the anhydride, indicates the presence of resin.

(2) *Raspail Reaction.* A piece of the paper is placed on a glass or porcelain plate, a drop of concentrated sugar solution applied and allowed to remain for a few minutes. The excess is then removed with filter paper, and a drop of concentrated sulphuric acid is applied to the spot of sugar solution. A raspberry-red coloration indicates the presence of resin.

Quantitative Determination of Resin. At least 5 grms. of the paper, cut from various places so as to be representative, are shredded into small pieces and the weight in a normal atmosphere obtained. The sample is placed in a suitable extraction apparatus, Soxhlet or Underwriter's, and extracted thoroughly with acidulated alcohol (90 c.c. of 95 per cent. alcohol, 0·5 c.c. of glacial acetic acid, and 9·5 c.c. of water). The extraction should be continued till the solvent comes over colourless, twelve syphonings at least being necessary.

If the paper contains nitrogenous sizing agents (such as glue and casein) in addition to the resin, the alcoholic extract must be freed from these by the following manipulation. The extract is washed into a beaker and evaporated to a few c.c. on a water-bath. The residue is cooled and taken up with 25 c.c. of ether, transferred to a separating funnel and washed with 150 c.c. of distilled water containing a small

quantity of sodium chloride to prevent emulsification. After separation of the layers, the water is run off into another separating funnel, and shaken with a further 25 c.c. of ether. The ethereal extracts are combined, and the whole further washed with successive quantities of 100 c.c. of water until a clear ethereal layer, separating sharply from the water, is obtained. Two washings at least should be given.

If any glue extracted from the paper should interfere by emulsifying with the ether, it may be eliminated by washing the combined ethereal extracts with a strong solution of sodium chloride, twice if necessary, before washing with distilled water as above.

The ethereal extract, purified as above, or the original alcoholic extract in the event of nitrogenous materials being absent, is transferred to a weighed evaporating basin and evaporated to dryness. The basin and contents are dried for exactly one hour at 100°, cooled in a desiccator, and weighed. The resin content is expressed as percentage on the weight of the sample taken. Two determinations should be made, and these should agree within 0·2 per cent.

Paraffin Wax, Oils and Fats. These substances may be determined by the extraction of 1 grm. of the sample, cut up into small pieces, in a Soxhlet or other suitable extractor with petroleum spirit. The solvent is distilled off, and the residue weighed. The percentage of paraffin wax, etc., is expressed on the weight of paper taken.

Acidity. Free acidity in paper may be determined by heating 10 grms. of the paper, torn up into small pieces, with distilled water on a water bath for an hour. The water is poured off and the paper washed repeatedly with distilled water, the washings being added to the extract. Extract and washings are placed in a porcelain basin. Beside this is placed another porcelain basin containing an equal volume of distilled water. Two drops of methyl orange indicator are added to each, and N/10 sodium hydroxide solution added from a burette to the paper extract until the tints in both basins are identical. The acidity should be expressed as percentage of sulphuric acid (H_2SO_4) on the paper taken.

Acidity due to the Excess Alum usually Present in Paper (*Paper Making*, p. 422). For some purposes—for example, the wrapping of metal goods—a paper specified "free from acid" is required. This is obtained by limiting the excess alum to a minimum, taking special care with the manipulation to get sufficient sizing effect. The best test for acidity of this nature is a highly dilute solution of potassium iodide and iodate in presence of starch. The solution is brushed on lightly and protected from sunlight. Paper containing the normal excess of alum will develop a blue-black stain

in a few seconds. A paper to comply with acid-free requirements should show no colour for five minutes.

Active Sulphur.—This also is injurious to metals, particularly silver. Paper intended for the wrapping of silver goods should be tested by the following method :

The paper (0·25 grm.) is either ground or thoroughly disintegrated in water and placed in a 500-c.c. flask having a neck about 2 inches long and 1 inch in diameter. Twenty c.c. of distilled water, 2 grms. of activated zinc cut into small pieces, and 10 c.c. of hydrochloric acid are added. A loose wad of surgical absorbent cotton, 1½ inches long, is inserted in the neck. A piece of hardened filter paper freshly moistened in 10 per cent. lead acetate solution is clamped in the neck by means of a piece of glass tubing loosely fitting it. Another wad of cotton wool is placed in the tube above the filter paper. Other flasks are fitted up in exactly the same way, but containing various small quantities of sodium thiosulphate solution instead of the sample of paper. All the flasks are placed on a water bath for an hour and the contents frequently agitated. The filter papers are then removed from the necks of the flasks and compared. The percentage of sulphur in the paper is found by comparing the depth of colour on the paper from the sample flask with that on the others from known amounts of sulphur evolved from the thiosulphate solution. The amount of sulphur is calculated on the weight of paper taken. For the wrapping of silver goods not more than 0·0008 per cent. of sulphur is permissible.

The reagents required for this determination are :

(1) Activated zinc. Small pieces of chemically pure zinc sticks, free from sulphur and arsenic, are covered with chemically pure copper sulphate solution containing 0·02 per cent. of copper (10 c.c. should be required for 1 grm. of zinc). After a few minutes, to allow for the deposition of copper, the zinc is washed with distilled water till free from zinc sulphate. The zinc may be re-activated several times.

(2) Concentrated hydrochloric acid free from sulphur and arsenic.

(3) Chemically pure lead acetate, 10 per cent. solution.

(4) Chemically pure sodium thiosulphate, 0·001 per cent. solution.

(5) Surgical absorbent cotton, sulphur free. This should be boiled in dilute sodium hydroxide solution and washed thoroughly with distilled water.

Colouring Matters.—*Organic Dyes.* An analytical examination for these is beyond the scope of this work. Tables for the recognition of coal-tar colours are given by H. A. Bromley (*The Paper Maker*, February, 1914, p. 224), and these should be

consulted if analytical work on the organic colouring matter is required.

Inorganic Pigments.—Smalts (potassium cobaltous silicate).—This usually occurs in high-class papers free from loading materials. It can be estimated with sufficient accuracy by determining the ash, deducting 1·5 per cent. as representing the natural ash, and taking the remainder as smalts.

Ultramarines.—These are of variable compositions. Their proportion is best determined by comparing the colour of the ash of a paper with known mixtures of ultramarine and china clay.

Chrome Yellow, Orange, etc.—The chromium and lead are determined by the usual methods of inorganic analysis. The results are calculated to the nearest indicated composition.

Prussian Blue.—This may be estimated through the iron content of the ash.

Miscellaneous Materials.—*Salicylic Acid.*—This is used as a preservative in wrapping papers intended for foods. It is extracted with petroleum spirit, the extract diluted with an equal volume of neutral 95 per cent. alcohol and titrated with *N* 10 sodium hydroxide solution, phenolphthalein being used as indicator. (One c.c. of sodium hydroxide is equal to 0·0138 grm. of salicylic acid.)

Carbolic Acid.—This is present in carbolised wrapping paper. As the bromine absorption method generally used for phenol is useless for commercial carbolic acid, the use of Muter's method is suggested :

From 10 to 20 grms. of paper, depending on the proportion of carbolic acid, are cut up into small pieces and extracted with 95 per cent. alcohol in a Soxhlet apparatus. The extract is transferred to a basin, mixed with about half its volume of 10 per cent. caustic soda solution, and the mixture evaporated to small bulk on a water bath. Tar oils and naphthalene, if present, separate out and can be filtered off. Th remaining liquid is transferred to a separating funnel, and hydrochloric acid added cautiously till the reaction is acid. Care must be taken not to allow the solution to become too hot during this process. Some brine is now added, and the funnel set aside. The tar acids rise to the surface and form a distinct layer. The aqueous layer is separated and rejected. The tar acids are dissolved in ether or petroleum spirit,

transferred to a weighed flask, the solvent evaporated, and the residue weighed. Only the acids so determined are effective as antiseptics, the tar oils being inert.

Specks in Paper.—The presence of spots or specks in paper not only spoils its appearance, but for some purposes renders it useless. For example, metal specks in insulating papers or hard particles of any description in paper to be used for gramophone record envelopes are serious flaws. It is desirable that the analyst should be able to determine their cause and suggest the appropriate remedy.

Rubber Specks. These find their way into paper from tyre fabric, waste paper containing rubber bands, or the elastic portions of underwear. They can be identified under a low-power microscope by stretching them with two dissecting needles. When burned they give the characteristic smell of burning rubber, and they are generally soluble in carbon tetrachloride.

Resin Specks.—Under the microscope these appear as translucent spots resembling resin. Sometimes they contain pieces of bark-like matter due to impurities in the resin size which have not been removed by proper filtration. In all cases enough resin is present to give the qualitative reactions for this substance.

Woody Specks.—These may be derived from splinters from a beater paddle, mechanical wood or cotton seed hulls. They can be identified microscopically, and all give the colour reaction of lignified fibres.

Iron Specks.—These may originate from parts of the paper-making plant or from iron buttons in rag pulp. The presence of iron can be proved either by the thiocyanate or ferrocyanide reactions.

Oil Spots.—These are usually soluble in ether or chloroform. If, however, they are due to heavy mineral oil containing gummy asphaltic impurities, they will not entirely disappear on treatment with the above solvents.

Colour Spots.—Badly ground colours give specks which can usually be identified by their particular colour.

Alum Spots.—These are generally pulverised by passage through the calender rolls. They are soluble in water, and the extract

gives an acid reaction with indicators. The reaction is best seen microscopically by placing a spot of the extract near a spot of the indicator and letting these run together.

Coal Particles.—These are easily mistaken for iron particles, and a test for this element should be made. In its absence, crushing of the particle under the microscope will generally be conclusive, owing to the characteristic manner in which coal particles shatter.

Button Specks.—These are derived from bone buttons crushed in the beaters. They are differentiated from alum spots by their insolubility in water and the absence of acid reaction with indicators.

Paper Specks.—These are due to the presence of old paper which has not been completely disintegrated. The spots can be characterised by pulling them apart with needles under the microscope, when their fibrous nature is evident.

Foam Spots.—These are caused by a foam bubble bursting on the partly formed sheet. The appearance is characteristic, being similar to a small round water-mark.

Knots.—These originate from rag stock fabrics with knotted threads. They are easily recognisable under the microscope.

Sand.—Particles of sand occasionally find their way into paper. During calendering they sometimes fall out and leave a hole. A particle of sand can be identified by its resistance to all acids except hydrofluoric.

Bronze Spots.—These arise from particles of bronze from beater bars, etc., and show a peculiar fern-like structure which may be confused with the growth of a fungus. Potassium ferrocyanide gives a chocolate colour due to the presence of copper in the bronze (*cf.* Stevens, *The Paper Mill Chemist*, p. 286).

Moulds and Bacteria.—Moist paper and pulp readily grow moulds, particularly if mechanical wood, casein or glue is present to afford a suitable nitrogenous soil. Moulds are easily recognisable under the microscope by their branching stems, and under good conditions by the spore-bearing organs resembling flowers.

Brown spots containing iron have been known to be the result of iron bacteria.

PHYSICAL TESTS

As this book is intended for the general practitioner rather than for the specialist, the question of physical testing is dealt with as simply as possible. To omit it altogether, however, is impossible, since the properties of paper cannot otherwise be fully evaluated. No more special apparatus than necessary is described.

Since practically all the physical tests applied to paper vary with the relative humidity of the atmosphere with which the material is in equilibrium, it is essential that these tests should be conducted under standard conditions of humidity. The results of numerous experiments indicate that the amount of moisture in paper, and those physical properties which are affected by moisture, bear no definite relation to the absolute humidity (weight of moisture per unit vol. of air). They are governed rather by the relative humidity, that is, the ratio of the amount of moisture in the air to that which it can hold when saturated at the same temperature. This is usually expressed in percentage relative humidity, a hundred times the above value. With constant relative humidity the effect of temperature is comparatively small. Control of relative humidity is essential, and temperature also should be maintained reasonably normal. For standard tests a normal atmosphere having a relative humidity of 65 per cent. and a temperature of 70° F. is recommended.

The most convenient method of obtaining controlled relative humidity in the laboratory is to place the material to be tested over a solution giving the required aqueous vapour pressure until it attains equilibrium. The solution should be placed in a shallow dish, and the substances to be tested supported over it on a wire gauze, the whole being enclosed in an air-tight container. The top of the container should not be more than 5 inches to 6 inches above the surface of the solution, and this should occupy most of the area of the bottom of the container. Such an apparatus can readily be built up from wood, sheet metal and wire gauze. The wooden box must be metal-lined. For very small quantities of material a glass desiccator will suffice.

Sulphuric acid of suitable strength constitutes a convenient

solution, since the aqueous vapour pressures of all strengths are accurately known.

For a container with metal parts, acid is not suitable, and glycerin may, with advantage, be substituted. A graph (Fig. 10) is appended, from which the relationship of various strengths of sulphuric acid and glycerin to relative humidity can be obtained.

Weight.—This is usually quoted in lbs. per ream of 480 sheets

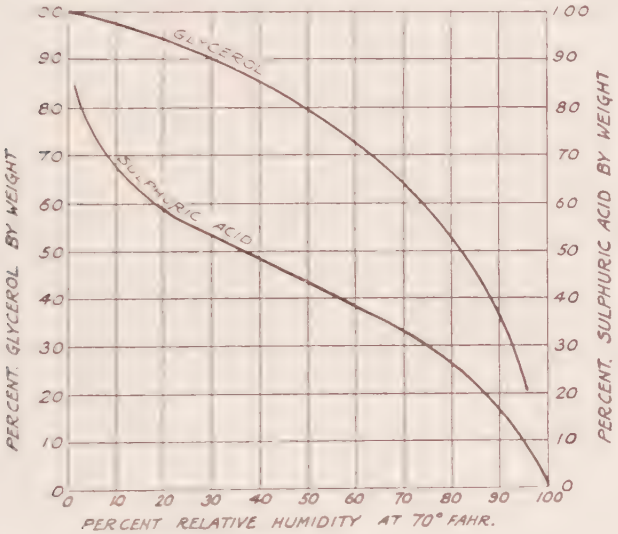

FIG. 10.—Graph showing Relationship of Sulphuric Acid and of Glycerin to Relative Humidity.

of a given size. Efforts are being made to standardise the ream at 500 sheets, in order to simplify calculations. The weight of a ream may be determined by weighing a sample of the paper, say, 5 inches by 10 inches, on an ordinary chemical balance sensitive to 0·001 grm., and calculating to weight per ream of any given size by using the formula :

$$\frac{N \times A \times t \times 0{\cdot}0022046}{(a)}$$

where A = area of sheet in ream, (a) = area of test piece, t =

weight of test piece in grms., N = number of sheets in ream, and 0·0022046 = conversion factor of grms. to lbs.

Weight is, of course, affected by the hygrometric state, and the weighing should be done under normal conditions. A number of samples should be drawn from a consignment so as to be thoroughly representative, and each should be tested, maximum, minimum, and mean values being given for the consignment. Variations of ± 5 per cent. from the specified weight are allowed in the paper trade.

Thickness.—For ordinary purposes the thickness of paper may be determined by means of a micrometer screw gauge with large

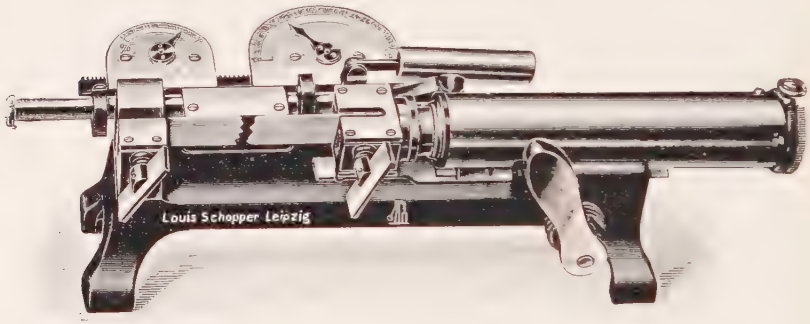

Fig. 11.—Small Schopper Tensile Testing Machine.

area flat ends, such as is supplied by Messrs. Brown & Sharp. More expensive instruments of the spring-activated dial type are preferable for more exact work.

Bulk.—The trade conception of bulk is represented by the ratio $\frac{T}{W}$, where T represents thickness and W the weight. T may be measured in thousandths of an inch (the measurement of four plies has been recommended (*Paper Making*, p. 402), and W in lbs. per ream.

Breaking Strain and Elongation.—Determinations should be made both along and across the machine direction of the paper. This is the direction in which the pulp flows along the wire on the paper machine. There are a number of machines on the market

for determining breaking strain and elongation ; of these, the small Schopper machine illustrated (Fig. 11) is recommended as being relatively inexpensive and very rapid in use.

Determination of Machine Direction. All machine-made papers show a greater strength and less elongation along the machine direction, while they show less strength and greater elongation

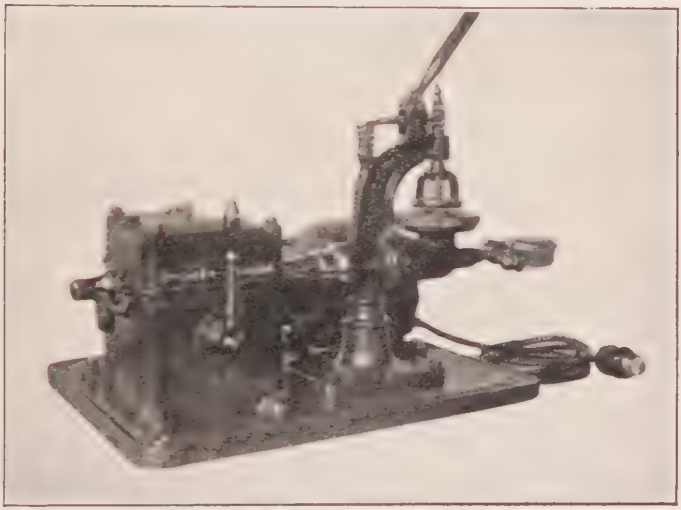

FIG. 12.—Bursting Load Testing Machine.

across it. The machine direction is most certainly determined by this test.

Two simple ways of obtaining the machine direction without special apparatus are :

(1) Float a specimen on water and note which way it curls. The axis of the curl is parallel to the machine direction.

(2) Hold two strips of equal dimensions, cut from the sheet at right angles to one another, in a vertical position. That which represents the machine direction will stand up the straighter.

Wire or Felt Side of Paper.—The wire side can generally be distinguished from the felt side by noting the marks of the wire on the surface of the paper. If the paper is plunged into water

for a moment and the excess blotted off, they will usually become more prominent, because the flattening effect of the calenders is undone. The method is often successful, even with coated papers.

Bursting Load.—For this determination a Mullen or Asheroft tester is required. Both machines are of American origin, but can be purchased through English agents. In the Mullen tester illustrated (Fig. 12), hydraulic pressure is exerted against a piece of paper, suitably clamped, through a circular rubber diaphragm

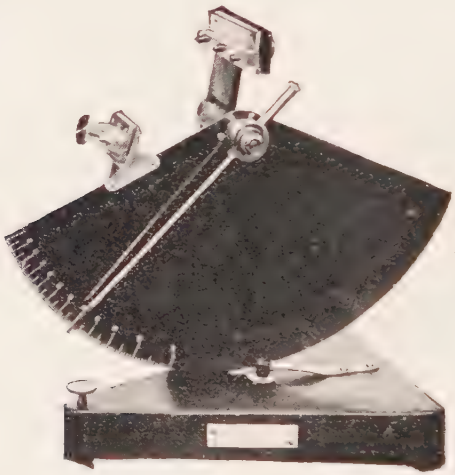

Fig. 13.—Elmendorf Tearing Strength Testing Machine.

until it bursts, and the bursting load is registered on a pressure gauge attached to the machine.

Folding Endurance Test.—The tests so far described serve to determine the strength and elongation of a paper, but these factors are insufficient to characterise a paper as regards its resistance to the mechanical damage it may meet in daily use. On this point information is best obtained by determining the number of folds backwards and forwards which a paper will stand before fracture. The standard machine for this purpose is made by Schopper and is illustrated in Fig. 14. Weak papers will stand

from one to twenty folds only, medium papers from twenty to 200 folds, and very strong papers from 200 to 1,000 and more folds.

Tearing Strength (Elmendorf). Further information on the properties of paper may be gained by determining its resistance

Fig. 14.—Schopper Folding Endurance Testing Machine.

to tearing, and an apparatus designed for this purpose by Elmendorf is illustrated (Fig. 13). The tearing strength is read off from the scale in grms. With weak papers several sheets may be torn at once.

Air Porosity. Paper being a discontinuous substance made up of an agglomeration of fibres, its properties are, to some extent, influenced by its closeness of texture. Type and degree of beating

affect this. On the one hand, it is possible to beat a paper so thoroughly that it resembles parchment, in which case its porosity to air is very low; on the other, to beat the same stock

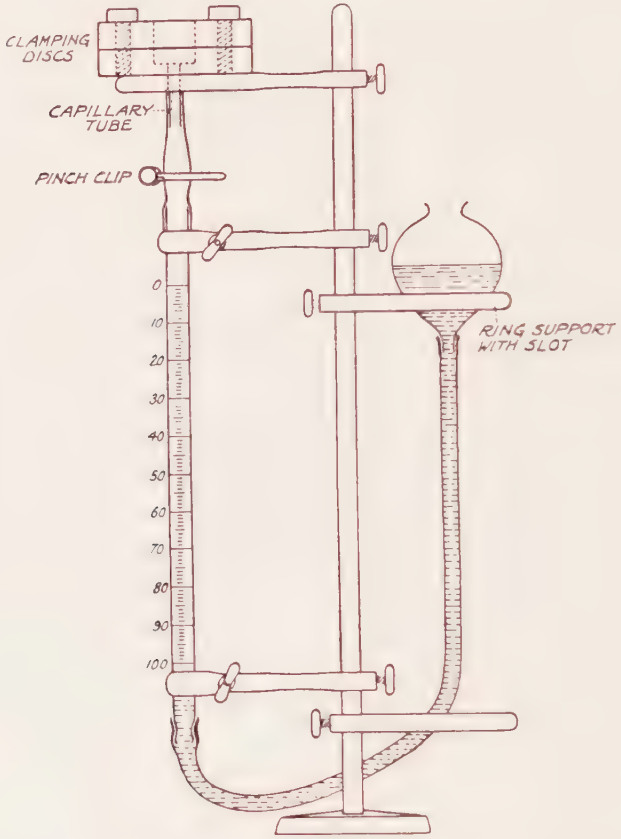

FIG. 15.—Apparatus for Measuring Air Porosity.

so lightly that its porosity is high. Super-calendering, sizing, or special treatments for the purpose of waterproofing or grease-proofing naturally also affect air porosity.

Several tests and forms of apparatus (*Paper Testing Methods*, p. 73) are available for the control of air porosity, the following test

being recommended because it necessitates only such apparatus as is available in any well-stocked laboratory :

The apparatus required comprises a 100-c.c. burette, the graduations of which, from 0 to 100 c.c., should measure approximately 22 inches, and two metal clamping discs having a 1-inch recess in the one and a 1-inch hole in the other, the inner sides of each disc being fitted with sheet rubber $\frac{1}{16}$ inch thick. The pinch-clip of the burette is connected with the clamping discs by a rubber tube having inside it a glass capillary tube of such a bore as will restrict the influx of air into the burette to 100 c.c. in fifteen seconds when no paper is inserted between the metal clamps. The arrangement of the apparatus is shown in Fig. 15. The pinch-clip of the burette is then opened, and the 300-c.c. flask is placed in the upper support. The flask should contain sufficient water, and the support be so adjusted that when the surface of the water in the burette coincides with the zero mark, the depth of the water in the flask is about 1 inch. The pinch-clip is closed when these conditions have been obtained. The strip of paper to be tested is clamped between the rubber-faced discs and the flask lowered to the bottom support, so placed that the surface of the water in the flask is 24 inches below the zero mark on the burette. The pinch-clip is opened, and the number of c.c. of air entering in fifteen seconds recorded. This gives a measure of the porosity.

Voids in Paper.—The determination of voids in paper by immersion of a weighed sheet, say, 4 inches by 4 inches, in an oil of known density, removal of surplus oil, and reweighing, yields further information regarding the properties of paper. This method is well known, but new light is thrown on the interpretation of results by A. S. Hammond (*Paper Trade J.*, February, 1929, 55). The effects of both beating and calendering are traced graphically, and suggestions made for the analysis and classification of paper. The original article is of considerable interest, but cannot readily be summarised.

Internal Sizing.—Paper is usually water resistant to a certain extent (*i.e.*, sized) by the incorporation of water-resisting substances, such as resin. The quantity of resin present is, of course, a determining factor. Different methods of treatment in the manufacture of the paper, however, cause such variations in the sizing effect, even when the same proportion of resin is present, that some direct test of sizing is required.

A method of procedure, simple and enjoying the prestige of custom, is to float the specimen on the surface of ink and to note the time taken for the first sign of the ink staining through. The longer the ink takes to come through, the better the sizing. The specimens are conveniently made up into small trays by folding in the manner taught to children in kindergarten schools. Flat-bottomed trays, with sides which prevent the ink from flowing on to the top surface of the paper, are formed.

Standard Ink.—A standard ink similar to that of the United States Government should be used (*Paper Testing Methods*, p. 89): This contains tannic acid, 23·4 grms.; gallic acid, 7·7 grms.; ferrous sulphate (crystals), 30 grms.; dilute (10 per cent.) hydrochloric acid, 25 grms.; phenol, 1 grm.; ink blue S. (British Dyestuffs Corporation), 3·5 grms.; with water to make up to 1,000 c.c. The ferrous sulphate is dissolved in cold water, the hydrochloric acid is added, and the tannic and gallic acids dissolved in warm water. The dye and phenol are also dissolved in warm water and added. Chemically pure reagents are used, and the dye must be tested with phenol to make sure that it does not give a metallic-looking film.

Work done by the American Bureau of Standards (*Paper Testing Methods*, p. 79), however, shows that the ink flotation method is erratic, and that the dry indicator method is to be preferred to it, and indeed to all others. This method depends on the fact that sugar gives an indication of transuded moisture, through the particles melting down to droplets long before the presence of water can be detected by any other simple means. The delicacy of the test is enhanced by adding a soluble dye to the sugar.

Various modifications of the method have been described. The simplest, consistent with a sharp end-point, is as follows:

A rectangular 80-mesh sieve, about 2 ins. by 3 ins., divided into three compartments by cardboard partitions, is prepared. A dry mixture of sugar and methyl green dye in the proportion of 50 : 1 is made up and placed in the centre compartment. The other two compartments are filled with a similar mixture of sugar and an insoluble pigment to give the same tint as the sugar dye mixture. The sample of paper to be tested is made up into a tray, as suggested in the ink flotation method,

and the sieve is dropped into its centre from a height of ½ inch. If the state of division of the particles of the mixture is right, three bands are deposited on the paper. The centre one contains sugar and dye, while the outer ones contain sugar and insoluble pigment. The tray is floated on water, and the tints in the three bands watched. The end-point is reached when the colour of the centre band sensibly increases in comparison with that of the outer two. The time from the floating of the specimen on water until the development of the end-point is recorded.

Factors necessary for accurate results are: Uniform temperature of water, a definitely standardised indicator mixture, and uniform conditions of humidity. The indicator mixtures should be kept in a desiccator when not in use.

Surface Sizing.—This has to do with the behaviour of paper towards writing ink. Fullest information on the subject is given by Hertzberg (*Papierprüfung*, p. 208), who advocates the following tests. In carrying them out, care should be taken that the relative humidity is fairly high (65 per cent. at 70 F.). Some papers give better results if the ink dries quickly, as is the case at low humidities, than if it dries slowly. If a paper is glossy on one side and smooth on the other, both sides should be tested. In giving an opinion the use to which the paper is to be put must, of course, be considered.

(1) Lines of different widths are drawn with a drawing pen, care being taken to make them as regular as possible. The points of the pen should be set for the desired width of stroke, and the pen filled with ink up to a certain mark. Each line drawn should be continued until the pen is empty. Successive strokes should be made at the same angle to the paper and at the same speed. Crossing of lines is undesirable, since the damage to the surface produced by one stroke may cause the second to penetrate. Lines of 0·5 mm. should be drawn to start with, and the width be increased by 0·25 mm. until the ink penetrates the paper. The width at which penetration just appears is the "critical width of stroke." An ordinary office paper giving 0·75 mm. "critical width of stroke" may be considered good. Envelope paper, which is to be written upon one side only, is satisfactory if the lines are sharp, even if they penetrate.

Tests of this type are usually comparative, so that, though simple, they are effective.

(2) When desirable, however, it is recommended that a supplementary test should be made by actual writing with an ordinary pen.

In such a case it is advisable that the same words, say, a name and address, should be used in comparison.

(3) A third criterion may be had by painting the paper with ink. This gives an indication of the uniformity of sizing. Any spots where sizing is poor owing to specks of filling material, etc., appear on the back of the paper as black spots.

(4) The effect of the pressure of the pen upon paper is best brought out by writing with a dry pen and subsequently floating the paper upon ink, with the side on which is the writing in contact with the ink. If the paper is not sensitive to pressure, the writing will remain invisible. If the paper is sensitive, the characters will become visible on the ink side. If very sensitive, they may even be seen upon the side not immersed.

(5) To evaluate the sizing of a paper as regards erasures, the paper should be torn so that a peeling effect is obtained, the internal substance of the paper being exposed. Lines should be drawn on the exposed part, and if they do not spread or penetrate, the paper may be considered good from the erasure point of view. At the same time, it must not roughen unduly during erasure.

(6) In dealing with writing papers on which printed lines, etc., appear — for example, account forms, ledger paper and the like — a complication arises owing to the printing. Test lines should be drawn over the printed lines, as well as on the free parts. It will often be found that penetration occurs at the intersection of the test and printed lines, whilst it does not appear on the plain paper. In this case it is the printing that is at fault rather than the paper.

Water Resistance.—This may be considered as a special case of sizing effect in which water is the penetrating liquid involved. Water resistance, or waterproofing, generally implies a longer period of transudation than is dealt with in sizing tests. The difficulty with water resistance tests lies in the detection of transuded moisture. This is overcome by the use of the Bureau of Standards ground-glass method (*Paper Testing Methods*, p. 82), which is as follows :

A glass cylinder, open and ground at both ends, is dipped in melted paraffin wax, drained for a second and applied to the specimen to be tested. The cylinder is kept in position by a weight until the wax has hardened. A sheet of ground glass is placed on a black surface, and the cylinder (with the specimen attached) placed thereon. A few inches of water are poured into the cylinder (the quantity is immaterial), and the time taken by the water to pass through the specimen is noted. The presence of transuded water is shown by a fugitive dark patch on the ground glass when the cylinder is lifted. This method is

peculiarly sensitive to variation of temperature, and comparative work should be done at a constant temperature as low as convenient.

Grease-proofness.—This property can be evaluated by deter-

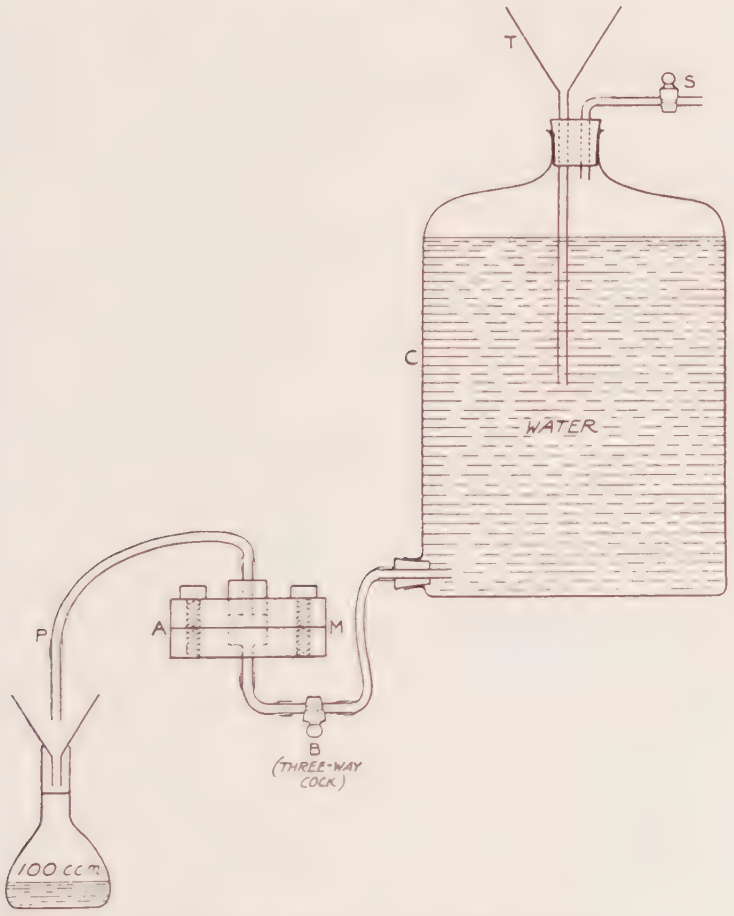

FIG. 16.—Apparatus for Testing Filter Paper.

mining the ease with which an organic liquid, say, turpentine, passes through the paper (*Paper Testing Methods*, p. 85). The same kind of glass cylinder as in the test for water resistance should

be used. The specimen is fixed to the cylinder by thick syrup or glue. Some turpentine, dyed a strong colour by a spirit-soluble dye, is put in a crystallising dish, and the bottom of the cylinder (with the specimen attached) immersed therein. The whole is placed on the top of a card with a circular hole in diameter slightly less than the cylinder. A mirror is fitted on a swivel underneath in such a way that a strong beam of light can be directed upon the hole in the card. A good light on the upper surface of the specimen is also required. The penetration of the liquid is indicated by the appearance of spots which look alternately dark and light as the mirror is oscillated.

Testing of Filter Paper.—It is frequently desirable to ascertain the rate of passage of water through filter paper. This can be done by an apparatus designed by Hertzberg (*Papierprüfung*, p. 208), or, in the absence of such special apparatus, by one easily constructed and utilising the clamping device described under "Air Porosity." This is attached to an aspirator bottle, as indicated in Fig. 16 (p. 305).

The stopcock is turned, so that A M and C are not in connection. C is filled with well-boiled distilled water through T, S being open. S is closed, B opened, and the water allowed to flow nearly up to the level A M, upon which it is shut off. A piece of filter paper is clamped in position between the two halves of A M and connection made between A M and C. Water flows out of pipe P, and the time taken to collect 100 c.c. is noted. The flow takes place under a constant head of water depending on the distance between the bottom of the funnel T and the tip of pipe P, 50 mm. being recommended for this distance. A number of samples, say, ten, should be tested and the average result taken.

The durability of filter paper can also be tested by increasing the head of water until the paper is ruptured.

The efficiency of filter paper for separating fine precipitates may be tested by mixing equal volumes of barium chloride solution (122 grms. per litre) with potassium sulphate solution (87 grms. per litre) both cold and hot. The cold mixture is filtered cold, and the hot mixture is filtered hot. The filter paper is supported in a glass funnel in the usual way. Exceptionally good filter

paper will give a clear filtrate in the cold test, whilst medium papers will give a clear one in the hot test.

Blotting Paper.—A blotting paper should "bulk" high and should be very absorbent. Absorbency may be determined in a number of ways.

(1) *Strip Method* (*Paper Testing Methods*, p. 89).—Strips of the paper, ½ inch wide and 6 inches long, are cut and hung up vertically, so that

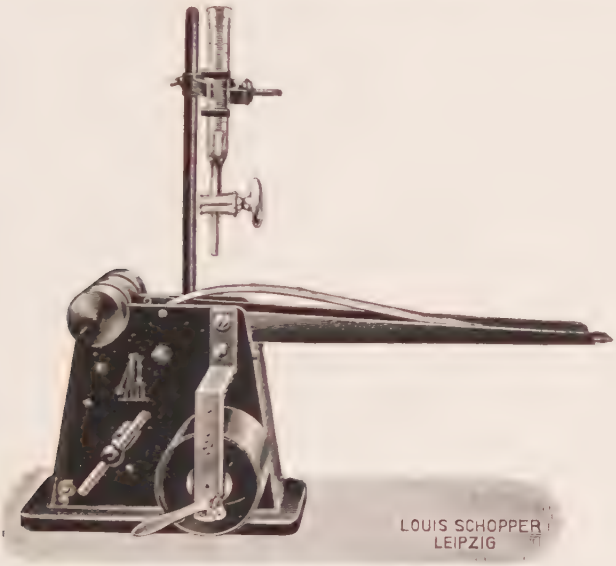

FIG. 17.—Dalen Blotting Paper Testing Machine.

their lower ends dip ¼ inch into water or standard ink (p. 302). A scale is set up alongside the strips, and a note is taken of the height to which the water or ink rises every minute for ten successive minutes. Five tests are made along the machine direction, five across it, and the average taken. The test may be repeated on the same specimens, in order to demonstrate the decreasing ability of the paper to absorb water or ink.

(2) *Blotting Test* (*Paper Testing Methods*, p. 90). Small pieces of paper, ½ inch wide by 4 inches long, are used to blot the same signature written with a stub pen and standard ink on bond paper, one signature at a time being blotted and the felt side of both blotting and bond papers

being used. The small size of the test specimen causes the blotting to be done on almost the same place each time. The number of signatures the paper will blot before the ink shows signs of spreading on the bond paper is recorded.

(3) *Zone Test* (*Paper Testing Methods*, p. 90). A piece of the blotting paper, 4 inches square, is placed, felt side up, on a piece of coarse wire gauze, and the whole supported upon the top of a beaker, an arrangement which prevents sagging during the subsequent operation. A burette containing standard ink (p. 302) is clamped above it, so that its jet is $\frac{1}{2}$ inch above the centre of the paper. One c.c. of ink is dropped upon the paper, 1 drop at a time, and each drop is allowed to be absorbed before the next is added. The time necessary for the absorption of 1 c.c. of ink is noted; also the diameter of the circular spot of ink immediately upon completion of the test. This spot consists of two zones: a dark one in the centre, and a lighter one round the periphery; in good papers the outer zone is not very marked and its area is small in comparison with the inner zone.

(4) A most elegant method has recently been introduced by Dalen, and an instrument for the purpose made by Schopper is illustrated (Fig. 17). A definite quantity of ink is allowed to fall from a burette on to a strip of well-sized writing paper. A strip of the same width from the blotting paper to be tested is brought into contact with the strip of writing paper under strictly regulated conditions, and the two are passed together through small rollers driven by a motor. The result is a long or short smudge, forming a tail to the drop of ink: the longer the smudge, the poorer the quality of the blotting paper.

Miscellaneous Properties

A number of other properties of paper, *e.g.*, durability (*Paper Making*, p. 408; *Paper Technology*, p. 148), opacity (*Paper Testing Methods*, p. 92), gloss (*ibid.*, p. 94), smoothness of finish (*ibid.*, p. 96), expansion and contraction (*ibid.*, p. 102), colour (*ibid.*, p. 104), stiffness (*ibid.*, p. 105), resistance to surface abrasion when wet (*ibid.*, p. 72), and presence of electrically conducting particles (*ibid.*, p. 97), may be determined. Space does not permit of these being dealt with in detail, but references to useful literature are given.

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CHAPTER IX

PETROLEUM AND ITS HYDROCARBONS

By J. G. King, Ph.D., F.I.C., and R. A. Acton-Taylor, M.Sc., F.I.C.

Elementary Analysis Composition : Proportion of main Groups — Petroleum Spirits New Methods of Separating Hydrocarbons. *Standard Tests :* Fractional and Engler Distillation. *Physical Constants and Tests :* Calorific Value Viscosity Flash Point Spontaneous Ignition Temperature Volatility Refractometry Colour Testing - Surface Tension—Miscellaneous Tests.

The evaluation of petroleum, and petroleum fractions and products, involves not only accurate chemical analyses, but also empirical tests the value of which depends largely upon strict adherence by the analyst to specified conditions. Tests of a physical character are similarly divisible into two groups one the accurate determination of physical constants, and the other embracing empirical tests similar to those of a chemical nature.

During the eight years' period of this review relatively little advance has been made in the application of pure chemical or physical tests, as these have all been clearly recognised for a much longer period. It is in the realm of empirical tests that the greatest advance is to be seen. The meaning of such tests is recognised only by analogy from previous experience, and the replacement of any such test by one of equal value which measures some definite chemical or physical property is greatly to be desired in oil technology. On this account the greatest advances have been made in the simplification and elucidation of empirical tests rather than in the methods of chemical and physical examination. In order to indicate the point from which advances have been made during the period under review, an endeavour has been made at the beginning of each section to describe the position during the year 1920.

Consequent upon a suggestion of the Institution of Petroleum Technologists a Standardisation Committee was appointed in May, 1921. Its membership represented all interested authorities, and comprised representatives of Government departments, petroleum companies and detached experts.

The American Society for Testing Materials and the American Bureau of Standards had already done much important work, which was duly considered, and largely incorporated into the methods recommended by the Standardisation Committee.

The outcome was that the Institution of Petroleum Technologists published a handbook of standard methods in 1924, and this passed into a second and much-augmented edition in 1929.

In order to avoid confusion in the review of methods applicable to special purposes, only the methods of examination have been grouped as such rather than under the headings of the type of substance to be examined. In certain cases this has not been possible, and these cases have been collected in a separate and final section.

ELEMENTARY ANALYSIS

The methods of elementary analysis of fuels were already fairly clearly defined before and during the war period, and it has been only in the perfecting of methods of determining sulphur, particularly in volatile oils, that any very great amount of investigation has been made.

Carbon and Hydrogen.—Liebig's method of analysis for carbon and hydrogen has been improved in accuracy in consequence of a research into the constitution of petroleum by Washburn, Brunn and Hicks (*Bureau of Standards J. Res.*, 1929, **2**, 467). Special precautions are taken for the purification of the oxygen and no rubber connections are used. An accuracy of ± 0·05 per cent. is claimed. The best temperature for the determination of carbon and hydrogen by the combustion tube method has been shown by King and MacDougall (*Fuel*, 1926, **5**, 33) to be 800°.

Sulphur.—One of the difficulties in Richardson's lamp method for determining sulphur was the absorption of sulphur by the wick, ascribed by Waterman and van Tussenbrock (*Brennstoff-*

Chem., 1928, **9**, 37) to the deposition on the wick of sulphur-containing asphaltic bodies in the case of heavier oils. Bowman (*J. Inst. Pet. Tech.*, 1921, **7**, 334) overcame the difficulty by burning the affected portion of the wick in a separate combustion apparatus, but Jackson and Richardson (*J. Inst. Pet. Tech.*, 1921, **7**, 26) removed it by replacing the wick by a bundle of glass capillaries "spread" by a central air-tube. The air does not pass through the spirit, so that fractionation is avoided, and, in addition, it assists the action of the capillaries by effecting a slight reduction in pressure above them. Above the capillaries is a mixing chamber. Other advantages have been claimed by the use of slight modifications. Korsakov (*Neft. Khoz.*, 1928, **14**, 68) surrounds the base of the absorption funnel with an inverted rim to collect condensed water (which may contain as much as 15 per cent. of dissolved sulphur dioxide). His lamp has a ground cover and stopper, and the titration is carried out electrometrically. Esling (*J. Inst. Pet. Tech.*, 1921, **7**, 83) gives a detailed description of a lamp, and Lomax and Bevan (*J. Inst. Pet. Tech.*, 1924, **10**, 914) have designed a lamp specially for benzol. Kennedy (*Ind. Eng. Chem.*, 1928, **20**, 201) has improved upon this by introducing a carburetting device. Davidson (*Gas J.*, 1929, **185**, 95) has designed a special burner in which the fuel is diluted with four volumes of sulphur-free methylated spirit. The products of combustion from 10 c.c. are burned during one and half to two hours, absorbed in neutral hydrogen peroxide solution, and titrated with *N* 2 sodium carbonate solution. It is claimed that this method is available for determining sulphur in heavier and crude oils, but Griffin (*Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 167) states that it may yield low results with heavy crude oils through incomplete oxidation of mercaptans. Helsinga (*Chem. Weekbl.*, 1925, **22**, 98), though realising that for kerosenes and light oils the lamp method is still the best, details an alternative combustion method. The oil (60 to 400 mgrms.) is burned in a current of purified air in a heated silica tube (40 cm. × 1 cm.) packed with quartz. The sulphur dioxide is absorbed in a 3 per cent. solution of hydrogen peroxide and is titrated with *N* 40 sodium carbonate solution. The results compare well with the bomb method (*vide infra*).

This method appears to bear considerable promise and is worthy of further examination.

The Eschka method, universally favoured for solid fuels, is approved for use with oils by the Italian Government, but de Fazi (*Ann. Chim. Applic.*, 1926, **16**, 405), not surprisingly, pronounces it as untrustworthy, giving lower results than the bomb method (*vide infra*). Another crucible method has been proposed by Hiller (*Z. angew. Chem.*, 1923, **36**, 25). Its outstanding advantage is rapidity. The fuel is mixed with 1 grm. of potassium chlorate and 10 grms. of sodium peroxide. The mixture is introduced into an iron crucible with a screw-on lid. After the combustion a special holder is necessary to remove the lid. The sodium sulphate formed is converted into barium sulphate for a gravimetric determination. For volatile oils a special iron tube with a ground-in stopper is used.

The use of a calorimetric or special bomb for sulphur determinations is now common. Griffin (*Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 167) has claimed that low results may be obtained, owing to incomplete combustion, particularly of mercaptans, with the formation of sulphonic acids, the barium salts of which are soluble in water. Heating beneath a reflux condenser with strong hydrochloric acid is a possible cure. King and Crossley (*Fuel.*, 1929, **8**, 544) have shown that, if certain precautions are observed, the bomb method is accurate even with substances containing 10 per cent. of sulphur.

A modification to achieve rapidity is due to Christie and Bisson (*J. Ind. Eng. Chem.*, 1920, **12**, 171). The sulphuric acid formed on combustion is converted into the benzidine salt, which is isolated and titrated with standard permanganate solution. Woodward (*Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 117) has suggested a method whereby enough oil is burned in the bomb to produce 0·03 to 0·25 grm. of sulphuric acid. Potassium iodide is added as indicator, the solution is evaporated to just less than 50 c.c., powdered aluminium is added in sufficient quantity (about 0·01 grm.) to decolorise the solution, and alcohol is added to give a solution of 50 to 70 per cent. alcoholic strength. The solution is titrated with *N*/50 lead nitrate solution (standardised against

N/10 sulphuric acid) until a permanent yellow colour results. The results by this method do not differ by more than 1·8 per cent. from the gravimetric (barium sulphate) method, and have the important advantage of speed.

Ormandy and Craven (*J. Inst. Pet. Tech.*, 1923, **9**, 133) have described a simple method for indicating the sulphur content of light spirits. A strip of electrolytic copper foil, 2 cm. × 1 cm., is suspended in the neck of a flask in which the spirit is distilled according to the Engler method. For 1 mgrm. of sulphur per 100 c.c., the copper blackens: it scales if the content is 2 mgrms. Spirits with less than 1 mgrm. of sulphur per 100 c.c. may be considered commercially free from sulphur. This method is described by Dunstan (*J. Inst. Pet. Tech.*, 1922, **8**, 378). The percentage of spirit distilled when blackening of the copper strip occurs is taken as the index of objectionable sulphur compounds.

Ormandy and Craven detail a method for determining free sulphur in oils:

> One hundred c.c. of the spirit or oil diluted with spirit are shaken with about 3 grms. of mercury in a dry bottle. When the sludge of mercuric sulphide has settled the whole is shaken with dilute hydrochloric acid (1 : 100) and is filtered through an asbestos plug. The plug is washed with dilute hydrochloric acid and transferred to a beaker. Potassium chlorate (0·5 grm.) and 25 c.c. of strong hydrochloric acid are added. The liquid is heated to expel chlorine, then filtered, and the sulphuric acid in the filtrate is determined by precipitation as barium sulphate.

Faragher, Morrell and Monroe (*Ind. Eng. Chem.*, 1927, **19**, 1281) detail a method of analysing light oils for sulphur and various sulphur compounds, to wit, hydrogen sulphide, mercaptans, organic sulphides and disulphides and thiophene. The hydrogen sulphide is precipitated by means of cadmium chloride (cadmium mercaptides are not precipitated). The total sulphur is now determined by the lamp method, and the elemental sulphur is removed by shaking with mercury and filtering, whereupon the sulphur is again determined by the lamp method; the difference gives elemental sulphur. The remaining oil is now divided into two parts. One is analysed for mercaptans by

dissolving in benzene and shaking with alcoholic sodium plumbite or basic lead acetate. With the former reagent the lead compounds are dissolved in the alcoholic layer, so that a lamp determination of sulphur on the residual oil will give (by difference between its result and that of the previous lamp determination) the mercaptans removed. With the latter reagent the lead compounds are dissolved in the aqueous layer, in which they may be determined by adding an excess of decinormal sulphuric acid and titrating those not converted into lead sulphate. The second portion is analysed by treatment with zinc and acid, when the disulphides are converted into mercaptans which may be then determined as detailed. Finally, sulphides and residual sulphur are removed from the oil by shaking with mercuric nitrate, and the sulphur in the residue is then determined by the lamp method and is calculated to the equivalent thiophene, which is not affected by any of the other reagents.

Nitrogen.—The determination of nitrogen in petroleum by the ordinary Kjeldahl method has been considered by Poth, Armstrong, Cogburn and Bailey (*Ind. Eng. Chem.*, 1928, **20**, 83) to be inaccurate. They recommend the use of a large excess of sulphuric acid with potassium sulphate, and with both mercuric oxide and copper sulphate added they consider the method to be more reliable.

Chlorine.—Bowman (*loc. cit.*) uses the lamp method of combustion for sulphur for the simultaneous determination of chlorine. The sulphur having been precipitated by barium nitrate solution, the chlorine is converted into silver chloride and determined by comparing its turbidity with known standards.

Matthews (*J. Ind. Eng. Chem.*, 1921, **13**, 325) determines chlorides in petroleum by mixing 500 c.c. with 125 c.c. of acetone and shaking with 1,375 c.c. of water, care being taken to avoid the formation of an emulsion. When the water-acetone layer has separated, 400 c.c. are drawn off and titrated with *N*/20 silver nitrate solution.

Lead Tetra-ethyl.—The advent of motor spirit "dopes" has led to the evolution of special methods for their accurate determination. So far the greatest attention has been paid to lead

tetra-ethyl, especially in consequence of its possible toxicity in exhaust gases.

The detection of its presence is simple by the method of von Fellenberg (*Z. Unters. Nahr. Genüssm.*, 1925, **49**, 173). The spirit is burned in a lamp with a 1-mm. wick, and the vapours are condensed on a water-cooled surface.

If the amount is to be determined, there are several alternative methods. Birch (*J. Inst. Pet. Tech.*, 1924, **10**, 816) determines lead as sulphate. The spirit is treated with concentrated sulphuric acid and potassium nitrate, and the lead sulphate separated in the usual gravimetric manner. Von Fellenberg's method is a similar one, the treatment being with sulphuric acid and with nitric acid or permanganate. Ferreri (*Chim. Ind. Applic.*, 1925, **7**, 625) prefers to separate the lead first by boiling 50 c.c. of the spirit with 50 c.c. of concentrated hydrochloric acid (sp. gr., 1·19) in a round-bottomed flask fitted with a reflux condenser. The lead chloride is recovered by partial distillation and precipitation with alcohol; it may be weighed as such, but greater accuracy is achieved by converting it into lead sulphate. Ormandy and Craven (*J. Inst. Pet. Tech.*, 1924, **10**, 954) use quite a different method. Chlorine is bubbled through 100 c.c. of the spirit: the precipitate (probably tri-alkyl plumbic chloride) is extracted with dilute hydrochloric acid. The solution is separated from the spirit, boiled for half an hour under a reflux condenser, and the lead is precipitated as chromate by the addition of sodium acetate and potassium dichromate. The solution is kept hot to coagulate the precipitate, which is then collected on to a Gooch crucible, dried and weighed. By this method lead tetra-ethyl gives results which are 7 per cent. low, whilst the tetramethyl derivative gives results 4 per cent. low. The authors think this may be due to the impurity of the lead compounds with which they started.

The X-ray is used by Aborn and Brown (*Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 26). The source is a Coolidge tube working at 35 kw. and 20 milliamp., and the radiations are passed through the spirit in a brass vessel with aluminium windows. The amount of lead tetra-ethyl is measured by the deflection of a galvanometer. There is a direct proportionality between the amount of lead tetra-ethyl and

the deflection of a galvanometer, in circuit with a Bragg type ionisation chamber.

COMPOSITION

Following the determination of the elements, the most definite analyses are those designed to measure the amount either of definite compounds or of the groups of compounds, such as aromatics, paraffins, etc. Water, though an impurity, may be included in this group as, unlike "carbon residue," it is a definite substance and capable of accurate measurement. In 1920 the determination of the amount of aromatic substances and of unsaturated hydrocarbons was commonly effected by washing with sulphuric acid of different strengths, and it is doubtful if the figures quoted were more than mere approximations to the true values. Recognition of this fact has occasioned a great deal of work since that time, and it has also led to methods for the determination of benzene, toluene, etc., in motor spirits as a result of greater insight into the behaviour of these substances, and of unsaturated bodies, as constituents of motor fuels. The advent of substitute fuels, such as benzol, alcohol, tetralin, etc., also at this time, has occasioned the finding of new methods for the analysis of mixed spirits.

The amount of individual investigation on these lines has been so great that it is possible to quote here only the most important advances in methods of analysis.

Water. The accurate determination of water has always presented considerable difficulty, owing to emulsification, and methods have multiplied accordingly. The method generally applied in 1920, and still in favour, was that of distillation with a solvent. This method has not been appreciably improved, and xylene is still the favourite solvent or "water-carrier," though Tausz and Rumm (*Gas u. Wasserf.*, 1928, **71**, 417) have recommended carbon tetrachloride as removing the fire-danger.

Determination by gravimetric means holds the promise of greater accuracy; two methods have so far been put forward. Clifford (*J. Ind. Eng. Chem.*, 1921, **13**, 628) passes dry air through the material under examination, collects the evaporated water in

calcium chloride, and removes all vapours remaining in the absorbent by a current of dry air. The method is intended for "gasolines and certain other substances." Reiner (*Elektrotechn. Z.*, 1925, **46**, 1447 ; *Chem. Zentr.*, 1925, **96**, [ii.], 2334) maintains the oil (naturally a heavier mineral oil) at 120° for three hours while compressed air is passed through the flask containing it. The absorbent is phosphorus pentoxide.

The methods in which the errors of measurement are reduced to a minimum are those in which water is converted into gas and measured. Losana (*Chim. Ind. Applic.*, 1922, **4**, 570) employs sodium or calcium amalgam, preferably the former, and has found that the method is accurate if the amount of water is small, but if it is large the volume measurement should be substituted by one of pressure. The accuracy of measurement of the hydrogen is discounted, however, by two sources of error : incompleteness of decomposition of the water, and the possible reactivity of nascent hydrogen towards reducible substances, particularly certain oxygen, sulphur and nitrogen compounds, and unsaturated bodies, any of which are likely to be present in petroleum and its derivatives. Taubmann (*Z. anal. Chem.*, 1928, **74**, 161) treats a pyridine extract of the oil with magnesium methyl iodide, measuring the methane liberated. The method requires no heating, and it is claimed that several determinations per hour are possible and that it gives excellent results. The difficulties attendant upon the hygroscopicity of pyridine must be considered.

Boller (*Chem. Ztg.*, 1926, **50**, 531 ; *Petroleum*, 1927, **23**, 146) passes the water vapour from the oil over calcium carbide and determines the acetylene as the copper derivative. The oil is heated at 130° to 140° in a bath, and a pure, dry inert gas (preferably hydrogen) is passed through it to a calcium carbide tube, 50 cm. long, arranged in an iron tube, and mounted in an oven. After one hour the tube is heated to, and maintained at, 180° to 200° for one hour. The acetylene is led into an absorbent made up with 10 c.c. of 10 per cent. copper sulphate, 4 c.c. of strong ammonia solution, and 3 c.c. of hydroxylamine hydrochloride diluted to 30 c.c. The precipitated copper acetylide is filtered off and washed with dilute ammonia, when it may be dissolved in

dilute sulphuric acid, converted into sulphide and weighed as copper oxide, or dissolved in acid ferric sulphate and determined by titrating the ferrous iron with permanganate. The results, it is claimed, are slightly low, though good even for moisture contents as low as 0·01 per cent.

Several physico-chemical and physical methods have been put forward. Wood and Neale's method (*J. Inst. Pet. Tech.*, 1925, **11**, 471) consists in distilling the oil with toluene or xylene into a measuring tube, immersed in a freezing mixture, until there are 20 c.c. of distillate. The critical solution temperature with aniline is then measured. The amount of oil distilled must be selected to give less than 0·5 c.c. of water, and the method is not suitable for such oils as petrol and kerosene, which will distil over with the xylene. Ormandy and Craven (*J. Inst. Pet. Tech.*, 1926, **12**, 89) mention that 1 per cent. of water causes a rise in aniline point of 6·2° for heptane and 5·6° for cyclohexane, and they, too, consider the method as suitable for the determination of water in certain substances. Pflug (*Chem. Ztg.*, 1927, **51**, 717) has put forward a modification of Oertel's method (*Chem. Ztg.*, 1920, **44**, 854). The temperature rise is observed when 25 grms. of oil are added to 10 grms. of a mixture of 2 parts of anhydrous magnesium sulphate and 1 part of quartz powder in a heat-insulated apparatus. For rises in temperature up to 13° the radiation losses are uniform, and the percentage of water in oils of mean specific heat 0·5 cal. per grm. may be calculated by the constant factor 0·6. Other factors are applicable for apparatus of different dimensions or for oils of different specific heats. If the moisture is more than 8 per cent. (giving a rise of more than 13°) the oil must be diluted with a dry oil.

Swetlow (*Neft. Khoz.*, 1924, **1**, 533; *Chem. Zentr.*, 1925, **96**, I., 805) reports upon several methods of determining moisture. The method of Hoffman and Marcusson (Martens, *Das Materialprüfungswesen*, 1912, 440), and centrifuging, he regards as quite trustworthy. Centrifuging is carried out in a 60-c.c. vessel at 900 r.p.m., 25 c.c. of oil being used with 25 c.c. of spirit and 0·3 grm. of calcium chloride. Drying a heavy oil on a water bath is regarded as undesirable, as is also von Lissenko's methods

diluting the sample with paraffin oil or light spirit, allowing it to stand at 70°, and then reading off the volume of water. The addition of cuprous chloride does not make this method valid. Tschernoschukov (*Izv. Teplotech. Inst. (Moscow)*, 1928, **10**, 11) has confirmed Swetlow's opinion of von Lissenko's method.

Proportion of Main Groups. The analysis of oils for their content of paraffinic, aromatic, naphthenic and unsaturated hydrocarbons is usually effected either by the action of sulphuric acid or by the "aniline point" method. In the former method, which is of very old standing, the unsaturated hydrocarbons are removed by solution in moderately strong sulphuric acid and the aromatic compounds by sulphonation with strong sulphuric acid or oleum, or by nitration.

The "aniline point" method is used for determining the proportions of aromatic and naphthenic hydrocarbons: it involves the determination of critical solution temperatures before and after removal of the aromatics. This, as a definite analytical procedure, really dates from the time of Tizard and Marshall's important paper (*J. Soc. Chem. Ind.*, 1921, **40**, 201), and seems to enjoy the balance of favour (*cf.*, Pritzker and Jungkunz, *Chem. Ztg.*, 1923, **47**, 313), although there are also one or two oxidation methods for unsaturated hydrocarbons.

The use of sulphuric acid for determining the amounts of unsaturated hydrocarbons has serious disadvantages to which the large number of modifications which have been proposed bear witness.

A symposium (*J. Inst. Pet. Tech.*, 1926, **12**, 48) on the determination of unsaturated hydrocarbons elicited many different opinions on the strength of sulphuric acid to be used. Lomax and Pemberton considered 80 per cent. acid too weak, and advised that the separate fractions of the spirit should be examined. Moore advocated acid of 90 per cent. strength. Garner, using 93 per cent. acid, observed that the aromatic compounds sometimes go with the unsaturated ones. Brame decided upon 84·5 per cent. acid, and in continuation (*J. Inst. Pet. Tech.*, 1926, **12**, 221), remarked that with 85 per cent. acid 30 per cent. of the aromatic hydrocarbons might be removed, while 88 per cent. acid removed

50 per cent. Nametkin and Robinson (*Neft. Khoz.*, 1929, **14**, 775) used 98 per cent. sulphuric acid at 0° for unsaturated hydrocarbons.

Detailed methods for the complete analysis of petroleum fractions have been given by Dănăila and others. Dănăila, Andrei and Melinescu (*Bul. Soc. Română. Stiinte*, 1923, **26**, [4–6], 3) determine the volume of unsaturated hydrocarbons (A_1) by shaking together 7 to 10 c.c. of the oil with three times its volume of 98·33 per cent. sulphuric acid for twenty to thirty minutes in a conical flask, with a ground-in stopper in its long neck, graduated in 0·1 c.c.: enough sulphuric acid of specific gravity 1·84 is added to float the oil up into the neck of the flask, and, after standing for 8 to 10 hours, the volume change of the oil is observed. In a fresh sample the olefines are removed by treatment with mercuric nitrate (method of Tausz and Wolf, *J. Soc. Chem. Ind.*, 1919, **38**, 889A) or ozone. The aromatic hydrocarbons (A_2), are determined in the residue by treatment with 98·33 per cent. sulphuric acid. If Ar and Ol are the percentages of aromatic and olefinic hydrocarbons respectively, $Ar = A_2(100 - A_1)(100 - A_2)$, and $Ol = A_1 - Ar$. If the amount of olefines is less than 5 per cent. the method is unsatisfactory, and Tausz's process is recommended, in which $Ar = A_2(100 - Ol)/100$.

A method tentatively tried for determining cycloparaffins involves dehydration over heated nickel. This method, according to Dănăila and Melinescu (*Ann. Mines Roumanie*, 1925, **8**, 6; *Chem. Zentr.*, 1925, **96**, [II], 1002), is applicable only to fractions volatile in steam and boiling up to 150°, and for higher fractions an oxidation method is advocated (*q.v.*).

Dănăila and Stoenescu (*Bul. Soc. Române Stiinte*, 1926, **29**, 23) determine cycloparaffins by dehydrogenating over platinised asbestos at 300° to 310° (method of Zelinsky and Pawlow, *Ber.*, 1923, **56**, 1249) and measuring the unsaturated hydrocarbons, repeating the process until the amount of unsaturated hydrocarbons is constant and then determining the amount of saturated aromatic hydrocarbons.

Pritzker and Jungkunz (*Chem. Ztg.*, 1923, **47**, 313) determine aromatic hydrocarbons by shaking oil with fuming sulphuric acid containing 4 per cent. of free sulphur trioxide. The flask, of

40-c.c. capacity, has a neck of 7·8-c.c. capacity, graduated in 0·1 c.c. The unsulphonated residue is separated by centrifuging. Error due to the solution of the non-aromatic portion of the spirit in the sulphuric acid does not occur if the spirit contains less than 20 per cent. of aromatic hydrocarbons; above this value, a correction is necessary.

Kuhn (*Chem. Ztg.*, 1929, **53**, 701) does not consider the method to be accurate, and prefers to treat the oil (10 c.c.) with 2 parts (20 c.c.) of fuming sulphuric acid (with 4·8 per cent. of free sulphur trioxide) in a 150-c.c. flask fitted to a burette, 60 cm. long, graduated in $\frac{1}{20}$ c.c. with oil containing up to 30 per cent. of paraffins. His results are higher than those of Pritzker and Jungkunz; above 30 per cent. the differences increase to a maximum at 50 per cent., after which they decrease. An accuracy of 2 per cent. is claimed for any mixture.

Kattwinkel (*Brennstoff-Chem.*, 1927, **8**, 353) adds phosphoric anhydride to the sulphuric acid to increase its action upon aromatic hydrocarbons, and boric acid to decrease its reactivity for unsaturated hydrocarbons. The former reagent is prepared by adding 30 grms. of phosphoric anhydride to 100 c.c. of sulphuric acid of sp. gr. 1·84; 30 c.c. of this acid are shaken with 10 c.c. of the spirit for five minutes, whereby the aromatic and unsaturated hydrocarbons are removed together and are measured as an increase in volume of the acid layer. If the temperature rises above 40° it is necessary to dilute with benzene. For unsaturated hydrocarbons alone 5 grms. of boric acid are dissolved in 100 c.c. of sulphuric acid.

Heilingötter (*Chem. Ztg.*, 1929, **53**, 79) uses fuming sulphuric acid with 4 to 5 per cent. of free sulphuric anhydride, and obtains good results with mixtures containing up to 99·5 per cent. of aromatic hydrocarbons. The liquid is shaken with 2 volumes of acid at not more than 50°. A refractive index of 1·4415 indicates complete sulphonation.

Aniline Methods.—The aniline-point method of Tizard and Marshall (*J. Soc. Chem. Ind.*, 1921, **40**, 20) for determining the percentages of aromatic hydrocarbons in oils of low boiling point was an outcome of the most convenient features in the work

of Chavanne and Simon (*Compt. rend.*, 1919, **168**, 1111; 1920, **169**, 185, 285) and of Thole (*J. Soc. Chem. Ind.*, 1919, **38**, 391r). Cherchevsky (*Ann. Chim. anal.*, 1921, **3**, 53) has claimed priority (1910) for Chavanne and Simon's method, which involves a determination of critical solution temperatures in aniline. Thole's method was densitometric.

Tizard and Marshall found that the difference between the temperatures at which homogeneity just occurred in a mixture of equal parts of aniline and the liquid under examination, before and after removal of the aromatic hydrocarbons (by sulphonation with 3 volumes of 98 per cent. sulphuric acid for half an hour), was proportional to—in fact, was almost a direct measurement of—the aromatic hydrocarbon content. Little error is introduced if the aromatic hydrocarbons vary over the range from benzene to xylenes, but the aniline must be pure.

Petroleum Spirits.—For analysing petroleum spirits there are several comprehensive methods, which include that of Tizard and Marshall. Ormandy and Craven (*J. Inst. Pet. Tech.*, 1924, **10**, 101) determine naphthenes by removing unsaturated and aromatic hydrocarbons with 98 per cent. sulphuric acid, and examining the residue by Tizard and Marshall's method, and by densitometric and refractometric methods. Densities at 20° are taken, and mean boiling points calculated by averaging the temperatures of the "drop" and "dry" points of the 10 per cent. fractions, as determined by the Engler method. The curve given by plotting these two properties is a straight line for naphthenes and paraffins. The density of a naphthene, however, is 0·1 greater than that of a paraffin of the same mean boiling point; hence the percentage of naphthenes in a mixture is 1,000 times the difference between its density and that of a mixture of paraffins of the same mean boiling point.

The refractometric method involves calculation (by the Gladstone and Dale formula) from observations on compounds of known composition. Out of nine mixtures examined by the three methods, in two cases one or other of the three methods gave a result which was seriously in error.

Egloff and Morrel (*Ind. Eng. Chem.*, 1926, **18**, 354) claim

concordant results for the following method of analysis of fractions up to 210° :

Five hundred c.c. of oil are agitated with 80 per cent. sulphuric acid, the volume decrease of the oil being added to the residue obtained on redistilling the washed oil to 210° (this residue represents polymerised products). The aromatic hydrocarbons are determined by treating 20 c.c. of the oil with 50 c.c. of a mixture consisting of 25 per cent. of nitric acid, 58 per cent. of sulphuric acid and 17 per cent. of water, the value being obtained by multiplying the number of c.c. of separated nitro oils by 4·3. Naphthenes are determined by the aniline-point method, and the paraffins by difference.

Garner (*J. Inst. Pet. Tech.*, 1928, **14**, 695) determines the composition of cracked spirits by the following procedure : The aniline point is determined, before and after removal of the unsaturated and aromatic hydrocarbons, by agitation with 2½ volumes of nitric acid at − 5 to − 10° ; the percentage loss is also observed. Five curves are constructed, according to whether 50 per cent. of the remaining oil distils at 95°, 105°, 115°, 125° or 135°, and from these curves and the aniline point the percentages of naphthenes and paraffins are determined (pure hydrocarbons having been used to establish the correctness of this procedure). If the iodine value was greater than 25, the aromatic equivalent of the aromatic and olefinic hydrocarbons was found by the aniline-point method. The difference between this and the percentage loss on treatment with the nitric acid, multiplied by 5·3, gives the olefines, whence the aromatic hydrocarbons are directly calculable. If the iodine value is less than 25 the olefines are obtained by dividing it by 2·8 for aviation spirit (mid-point of distillation 95°), 2·7 for No. 1 spirit (mid-point 105°), and 2·6 for other petrols. By subtracting 40 per cent. of the aromatic hydrocarbon content from the total aromatic equivalent, as determined by the aniline point method, the nett content of aromatic hydrocarbons is obtained. The second method renders unnecessary the determination of the volume loss on treatment with nitric acid.

As an alternative to aniline, Aubert and Aubrée (*Compt. rend.*, 1926, **182**, 577) have suggested benzyl alcohol. By using both liquids, analytical results are obtainable by the solving of simul-

taneous equations—a procedure which avoids the necessity of removing saturated bodies by nitration.

New Solvents for Separating Hydrocarbons.—Methyl sulphate has long been used to distinguish paraffins and naphthenes from aromatic and unsaturated hydrocarbons. Taylor (*Ind. Eng. Chem.*, 1927, **19**, 76) has employed ethyl sulphate instead, with better results.

Göhre (*Petroleum*, 1927, **23**, 73), after investigating various solvents for this purpose, has found that lævulinic acid gives theoretical results up to 90 per cent. of aromatic hydrocarbons, and is recoverable by extraction with water. It should be used in the proportion of $3\frac{1}{2}$ to 1 for light and medium oils, and 2 to 1 for viscous oils. Phenylhydrazine is suitable for some oils, but not for light oils. Glycol monacetate (used in the proportion of 3 to 1) gives reliable results for light and medium oils; for heavy oils it may be used in the proportion of 2 to 1, but if the aromatic hydrocarbon content is high, cooling with ice is desirable. Furfuraldehyde (equal proportions) is suitable for all oils, but must be used at $-10°$.

Oxidation Methods.—An oxidation method for unsaturated hydrocarbons is suggested by Dănăila and Melinescu (*Ann. Mines Roumanie*, 1925, **8**, pp. 6; *Chem. Zentr.*, 1925, **96**, [II], 1002). A graduated burette containing an internal side tube is connected with the upper end of a conical separating funnel, 50 to 60 c.c. of 30 per cent. sodium hydroxide solution are run into the funnel, and then about half of it is run out, so that 25 c.c. of oil are drawn in from the burette. The whole is cooled to -10 to -15; oxygen containing 1 to 3 per cent. of ozone is passed in for six hours at the rate of 5 to 6 litres per hour. After twelve to fourteen hours the alkaline layer is drawn off, and the oil is washed four times with 30 per cent. caustic soda solution, three times with distilled water, once with 15 per cent. sodium hydrogen sulphide solution, and once with distilled water. The oil is then forced back into the burette and measured after three hours' standing. The accuracy of this method is affected by the presence of sulphur or nitrogen compounds and of suspended solids.

Oxidation by nitric oxide is suggested by Smirnov (*Neft. Khoz.*,

1928, **18**, 217), who determines unsaturated hydrocarbons in cracked spirits by adding them, drop by drop, to liquid nitric oxide at − 15 to − 20°. The residual oil is made alkaline with sodium hydroxide and distilled in steam. The distillate is dried, filtered through silica gel (which has been washed with ether), and the decrease in volume of the oil (after distilling off the ether) gives the volume of unsaturated hydrocarbons.

Nitration Method.—A nitration method due to Manning (*J. Chem. Soc.*, 1929, 1014) has the merit of employing very small quantities (0·2 to 0·5 grm.). The oil is vaporised in a current of air and passed through a solution of 10 per cent. of nitric acid (or 16 per cent. of potassium nitrate) in concentrated sulphuric acid contained in a bubbler. The increase in weight of the bubbler gives the total of unsaturated and aromatic compounds. The acid solution is heated for two or three hours on a water bath to complete the nitration, poured into an excess of water, and the nitro-compounds extracted with benzene and weighed. To obtain the amount of aromatics a conversion factor of 0·452 is used.

Definite Substances.—For the detection of certain substances in motor fuels Formánek (*Chem. Ztg.*, 1928, **52**, 325, 346) has devised a comprehensive series of tests covering petrol, benzene and its homologues, ethyl alcohol, ether and tetralin. The aromatic compounds are coloured red by the vat dyes, Algol red BTK and 2G, and rose-red by lake-red Ciba B, whereas the other constituents remain colourless. The aromatic hydrocarbon content of a petrol may thus be measured by comparing it with standards of known aromatic hydrocarbon content. Aniline blue 2B is insoluble in water, benzene and the other hydrocarbons, but colours such oxygenated compounds as alcohols, aldehydes and ketones. Ether may be detected by the perchromic test. A fuel with less than 20 per cent. of aromatic hydrocarbons may be tested for petrol by warming with aniline, when the petrol separates on cooling. Alcohol may be determined by shaking the spirit with aqueous fuchsin solutions and measuring the decrease in volume of the colourless layer. Tetralin may be detected by distilling the oil to 200° and using Algol red BTK to test the residue.

Schwarz (*Chem. Ztg.*, 1922, **46**, 401) has devised a simple method

for detecting benzene in petrol. A solution of equal parts of aniline and 95 per cent. alcohol is added to two and half times its volume of petrol. The aniline separates if there is no benzene, but remains in solution if the oil contains 5 per cent. of benzene. The fraction 80° to 100° may be specially tested.

Kattwinkel (*Chem. Ztg.*, 1925, **49**, 51) uses sulpho-acetic acid as a solvent for benzene in petrols: 100 c.c. of acetic anhydride (free from homologues) are heated at 85° with 8 c.c. of sulphuric acid until the liquid gives no turbidity with barium chloride solution. Eleven c.c. of this reagent are used with 10 c.c. of spirit, and the volume decrease of the spirit represents benzene. The method was found not to be applicable if the amount of benzene exceeded 50 per cent.

COMPOSITION: STANDARD TESTS

Distinct from the above methods of determination of definite substances or groups of substances, there are a number of tests the value of which has become known only by the correlation of results to the performance of oils in their various uses. In such tests observance of procedure is all important, since the substance or property measured is affected by the technique used. The value of tests such as this is often doubtful, and this doubt has been the cause of many attempts to improve upon the tests available.

Carbon Residue. The amount of carbon residue which remains on the heating of an oil under standard conditions is open to wide variation, not only according to the method, but also between repeat experiments by the same method. The only means of obtaining approximate agreement is by very strict observance of the conditions laid down.

The Conradson Test. The Conradson test is one which is standardised in America and varies somewhat from that adopted in this country.

Schultz and Kohout (*Petroleum*, 1927, **23**, 554) have found large experimental errors when using 10 grms. of oil, and recommend, instead, the use of 2 grms. in a Rosenthal No. 4 porcelain crucible placed into the apparatus in the cold. The whole is heated for

twenty minutes with a Tirrill burner having a 20-cm. flame, the iron crucible being fixed 5 cm. above the burner.

Piotrowski and Winkler (*Przemysl Chem.*, 1928, **12**, 573) have observed that, for lubricating oils, the Conradson coke number is never greater than unity if the oil is a distillate. Fatty acids up to 10 per cent. have no effect on it; naphthenes and aromatic hydrocarbons and vacuum distillates have a lower Conradson number than oils containing unsaturated hydrocarbons. Ries (*Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 187) adds that neither high wax content nor lack of volatility increases the Conradson number; in fact, removal of wax generally raises the number; wax itself leaves no carbon residue.

Cold Test or Pour Point.—This test has consisted essentially in the observation of the behaviour of an oil when subjected to a freezing mixture and is entirely qualitative. A suggested improvement upon the method is due to Lichthardt (*J. Ind. Eng. Chem.*, 1921, **13**, 145), who freezes the oil in an inclined glass tube immersed in an acetone and carbon dioxide freezing mixture, attaches one end of the tube to an air supply at 16 inches pressure, and allows the temperature to rise slowly. The "cold test" is taken as the temperature at which oil is ejected from the tube.

Asphaltum.—The methods of determination of hard and soft asphaltum, involving separation with petroleum spirit or ether-alcohol, have not been altered appreciably, but the fact that the methods are dependent to some extent on insolubility in a variable solvent accounts for vagaries in the results, and this will greatly reduce their value.

Differences due to the use of different petroleum spirits are pointed out by Evans (*J. Inst. Pet. Tech.*, 1923, **9**, 384). He finds that results are low if the oil contains aromatic hydrocarbons, or if the boiling range of the petroleum spirit is too high (< 40 is best). This latter observation is confirmed by Bourgour (*Bull. Féd. Ind. Chim. Belg.*, 1927, **6**, 201). In addition, the asphaltum sometimes contains paraffin wax, and repeated extraction with absolute alcohol is recommended by Holde (*Petroleum*, 1926, **22**, 789) to correct this.

Realising this difficulty in the use of organic solvents which are

affected by the oil under examination. Marcusson (*Chem. Ztg.*, 1927, **51**, 190) has suggested an ethereal solution of ferric chloride as a precipitating agent :

To 5 grms. of the oil dissolved in 50 c.c. of ether are added 5 c.c. of a 5 per cent. ethereal solution of ferric chloride. The precipitate is washed, extracted with boiling ether, and dissolved in warm chloroform. The solution is agitated with 5 c.c. of dilute hydrochloric acid, then with 5 c.c. of water, evaporated, and the residue dried at 105° and weighed.

Resins. Borodulin (*Petroleum*, 1927, **23**, 1515) prefers Akzy's method—diluting the oil with petroleum spirit, extracting with 95 per cent. sulphuric acid, and reading the increase in volume of the acid layer. The method of Armani and Rodano (*Chim. Ind. Applic.*, 1920, **1**, 45) Borodulin regards as unreliable.

Potential resins are determined by Jacqué (*Compt. rend.*, 1929, **189**, 486) by drawing air through the boiling spirit under a reflux condenser. The residue left on evaporation is dried at 105° and weighed.

Coal Tar.—The presence of coal tar and its fractions in oils is detected by Sauerbier (*Chem. Weekbl.*, 1927, **24**, 348) by a diazo test, but Nellensteyn and Sauerbier observed that if colophony is present the test is unreliable, and Millon's reagent should be used.

About 10 grms. of the oil are boiled with 25 c.c. of normal sodium hydroxide solution, the liquid is filtered, slightly acidified with nitric acid, and 5 c.c. of Millon's reagent added. If a coal-tar product is present a colour will develop after thirty minutes' heating on a water bath (*cf.* Chapin, *J. Ind. Eng. Chem.*, 1920, **12**, 771).

Unsaturated Hydrocarbons; Iodine, Bromine and Oxygen Values. Comparison of Reagents.—There are three methods of outstanding importance for the determination of iodine values : those of Hanus (*Z. Unters. Nahr. Genussm.*, 1901, **16**, 913), von Hübl (*Dinglers polyt. J.*, 1884, **253**, 281), and of Wijs (*Ber.*, 1898, **31**, 750). The methods all date back some considerable time, and, though their respective merits are much debated, all three are still widely employed. For petroleum compounds the Hanus method seems to hold the balance of favour (*cf.* Oils and Fats, p. 68).

As Johansen (*J. Ind. Eng. Chem.*, 1922, **14**, 288) has indicated,

iodine reacts by substitution as well as by addition. The extent of this substitution determines the error in the determination. The total iodine entering into reaction is equal to twice the iodine substituted, plus the iodine added, and the last factor only is a measure of the unsaturation.

Faragher, Gruse and Garner (*J. Ind. Eng. Chem.*, 1921, **13**, 1044) have found that the Hanus and Wijs reagents give the same values for amounts less than 0·1 grm. of oil. The Hanus method gives the true unsaturation value, and is not affected by small variations in the bromine excess. All cracked spirits contain diolefines, which may be detected by the shape of the curves given by iodine value against time of reaction, or iodine value and quantity of substance used with 25 c.c. of reagent.

Kawai (*J. Chem. Ind., Jap.*, 1922, **52**, 406) has investigated the effect of concentration, temperature and time, using all three reagents. He finds that the values descend in the order: Wijs, Hanus, Hübl, Holde.

Werner and Tacke (*Chem. Umschau*, 1922, **29**, 185) regard the Hanus reagent as preferable: it gives results in close agreement with the Hübl reagent, but it is more easily prepared, more stable, and more rapid in its action. The Hübl-Waller and Wijs reagents they consider to give anomalous results. In contradiction to this, Gane, Schwartz and Zilisteanu (*Bull. Soc. Chim. Romînîa*, 1924, **6**, 71) prefer the Wijs reagent. For cholesterol and phytosterol, Holde, Werner and Tacke found that the Hubl reagent gave consistent, though slightly high, values: Wijs' reagent gave very high values. If a similar difference is observed for mineral oils, the presence of these compounds is suggested.

Morrell and Egloff (*Ind. Eng. Chem.*, 1925, **17**, 1259) have used the Hanus method on a 1 per cent. solution of the oil in chloroform, the reaction being effected in the dark. An increase in the proportion of reagent caused an increase in the iodine value. They differentiate between added and substituted iodine, and observe that the amount of absorption in sulphuric acid is no measure of unsaturation.

Margosches, Krakowetz and Schnabel (*Petroleum*, 1929, **25**, 1179) have carried out comparative determinations with the Hübl

method and a method in which the oil is emulsified with water and treated with alcoholic iodine (Margosches, Hinner and Friedmann, *Chem. Ztg.*, 1924, **48**, 389; *cf.* Oils and Fats, p. 70). Concordant results were not obtained with heavy fractions of oil, but an improvement was effected by removing asphalt. They determined the conditions necessary to make the newer method agree with that of Hübl.

Bromine Values.—Zdárský (*Chem. Obzor.*, 1929, **3**, 165, 205; *Chem. Zentr.*, 1929, **1**, 1772), investigating bromine values, found that they vary according to the method, and even with standardised conditions give only relative values.

Frances (*Ind. Eng. Chem.*, 1926, **18**, 821) has determined bromine values by shaking the oil with potassium bromide and bromate in slightly acid solution. He claims that bromine is fixed at the double bonds, and that substitution is reduced to a minimum. Bacon (*Ind. Eng. Chem.*, 1928, **20**, 970) has used a similar method for lubricating oils. The oil is dissolved in benzene and added to 10 per cent. sulphuric acid. Small quantities of *N*/2 sodium or potassium bromide and bromate are added, with shaking, until free bromine is evolved, when the determination is completed iodometrically.

Oxygen Value.—Nametkin and Abakumovsky (*J. prakt. Chem.*, 1927, **115**, 56) have examined the sources of error in the determination of oxygen values by treatment with perbenzoic acid in chloroform. They found it necessary to determine a blank on the decomposition of perbenzoic acid in chloroform; to use 10 c.c. of semi-normal perbenzoic acid (or a 200 per cent. excess) and not more than 0·3 to 0·4 grm. of the oil; to carry out the reaction in the dark at 9° to 12° for forty to forty-eight hours; and to prepare the perbenzoic acid two days before use. The oxygen value, which should be equivalent to the iodine value, is the quantity of active oxygen taken from the perbenzoic acid to react with the unsaturated hydrocarbons; they devised a "saturation coefficient" $= \frac{100\ \text{O.V.}}{16} - \frac{100\ \text{I.V.}}{254}$. It sometimes happens that the oxygen value is greater than the iodine value, owing to the more drastic action of iodine.

FRACTIONAL AND ENGLER DISTILLATION

The Engler method of distillation still remains in favour as the best method of rapid fractionation, but the errors to which it is subject could well be reduced by closer standardisation of conditions. Its value as a means of assessing the suitability of petrols in engines is questioned by Stevenson and Babor (*Ind. Eng. Chem.*, 1927, **19**, 1361) on the ground that the vaporisation in the engine is not a fractionation, and they prefer a dew-point method.

In investigating thermometric lag, Ormandy and Craven (*J. Inst. Pet. Tech.*, 1924, **10**, 342) have recommended the use of a copper-eureka thermo-couple, in that it shows no lag, and therefore removes one of the greatest errors in fractional distillation.

Index Numbers.—A new departure in the evaluation of motor spirits is the adoption by Gruse (*Chem. Met. Eng.*, 1923, **29**, 970) of an index number obtained by adding together the temperatures at which every 10 per cent. of spirit distils over. This represents the volatility under test conditions and varies from 612 to 967 for different spirits; a low number expresses high volatility and good starting and accelerating properties, to which it bears a closer relation than the 85 per cent. point on Wilson and Barnard's dew-point curve (*J. Ind. Eng. Chem.*, 1921, **13**, 906).

Ostwald's index number (*Petroleum*, 1925, **21**, 1323) has recently commanded greater attention. His index number is a similar average boiling point obtained by calculating the algebraic mean of the temperatures at which 5, 10, 15 . . . 95 per cent. of spirit distil. For mixtures the index number may be calculated by the rule of mixtures from the proportions of the constituents and their average boiling points. Ostwald adds (*Petroleum*, 1926, **22**, 850) that the index number may be obtained from the boiling-point curve by drawing a straight line at right angles to the temperature axis, so that the area on either side of this line is equal to that on the other; the temperature of its point of intersection with the graph is the index number. The sharpness of fractionation may also be computed from the graph by drawing a line cutting the curve in at least two places, so that the area enclosed between the curve and the line below is the same as the area enclosed

between the curve and the line above. The inclination of this line to the horizontal is the fractionation number of the spirit; besides characterising the spirit, it is of assistance in measuring the efficiency of a fractionating column.

Nowosielski (*Przemysl Chem.*, 1929, **13**, 16) has approved this method of evaluation, and adds that the rule of mixtures permits of the calculation of the average boiling point with an error of less than 1·5 per cent. There is one dissentient, Kroch (*Petroleum*, 1926, **22**, 1245), who asserts that the method is not new and is misleading. Ostwald (*Petroleum*, 1927, **23**, 445), refuting Kroch's criticisms, claims an extension of the reliability of the method even for light spirit and benzol mixtures, and says that it is useful for indicating the boiling-point depression in alcohol mixtures.

Vacuum-Assay Distillation Test. As an alternative to the Engler distillation, Peterkin and Ferris (*Ind. Eng. Chem.*, 1925, **17**, 1248) have described an apparatus for fractionally distilling 100 c.c. of lubricating or other heavy oil from a 250-c.c. Claisen flask under a constant pressure of 10 mm. of mercury. This "vacuum assay distillation test" should form a valuable extension of Engler's fractional distillation.

PHYSICAL CONSTANTS AND TESTS

The general tendency since 1920 has been to increase the number of physical tests carried out, in particular those connected with the interpretation of engine performance in terms of laboratory values.

Calorific Value. The bomb calorimeter is still the only reliable instrument for oils of low volatility. The difficulty of dealing with volatile oils still exists. The gelatin capsules proposed by Barsky (*J. Ind. Eng. Chem.*, 1920, **12**, 77) do not seem to have found general favour. As pointed out by Cantoni (*Chem. Ind. Applic.*, 1926, **8**, 119), the difficulty lies only in the variable moisture content of the gelatin. The use of glass bulbs or of absorbents such as kieselguhr is also unsatisfactory. The only method which can be successful is one which will retain the vapour within the

crucible and so avoid detonation or ignition. Fuels which contain certain unsaturated hydrocarbons are stated (*Royal Aircraft Establishment Rept.*, No. HG 410; *J. Inst. Pet. Tech.*, 1921, **7**, 339) to display a tendency to detonate on ignition, and this is not prevented by dilution with xylene.

The most satisfactory method of dealing with volatile oils is to employ a flow calorimeter, as suggested, notably, by Moss and Stern (*Engineering*, 1922, **114**, 729). A new variation of this principle has been put forward by the Director of Fuel Research (*Annual Report*, 1929), in which the fuel is burned in a special burner in a Boys gas calorimeter. The only source of error is slight incomplete combustion.

A formula for the calculation of the calorific value of liquid fuels has been put forward by Morpurgo (*Mitt. Staatl. Tech. Versuchsamts*, 1921, **10**, 97; *Chem. Zentr.*, 1924, **95**, [I], 271). In the equation

$$\text{C.V.} = \frac{(11{,}200a + 10{,}300b + 8{,}140c + 2{,}500s + 10{,}000d)}{100}$$

a represents the fraction up to 110° obtained by Engler distillation; b, the fraction from 110° to 310°; c, the coke; s, sulphur; and d, the residue less coke and ash.

Each constituent is multiplied by its average heat of combustion.

Viscosity.—Studies of the viscosity of petroleum products are of interest chiefly with regard to lubrication, although in fuel oil specifications an arbitrary value is stated, to ensure that the oil will remain pumpable on cooling.

At the beginning of the period under review, the general practice was to determine an empirical value for the viscosity of an oil by employing a viscometer, such as that of Redwood, in which the rate of flow of a measured volume of the oil through an orifice was compared with that of a "standard" oil. The evaluation of oils by the obtaining of absolute values for viscosity was only in its early stage, and practically no attempt had been made to correlate viscosity determinations with the property which they were designed to measure, namely, lubrication. Since that time absolute measurement of viscosity has become more common, and

improvements have been made in the old methods, but even to-day it cannot be said that viscosity measurements by themselves offer any real indication of the value of an oil as a lubricant.

Work which has been done in improving the older methods of viscosity measurement is, briefly, as follows :

In the Engler viscometer one source of error is that the oil flowing out through the capillary exposes the bulb of the thermometer. Schluter (*Chem. Ztg.*, 1927, **51**, 565) has proposed the use of a thermometer with the bulb bent at right angles to the stem, so that it lies just above the floor of the oil container. A second thermometer is retained in the usual position to close the capillary before and after the oil has flowed out ; to maintain the oil at 50° the bath temperature has to be 50·3°.

Conversion Factors.—Bleyberg (*Petroleum*, 1928, **24**, 1416) has proposed shortening the time necessary for determining viscosities by the Engler and Holde instruments. These deliver 200 c.c. and 100 c.c., respectively, but if only a small volume (say, 10 c.c.) is timed, the following factors enable calculation to be made to the full time.

Engler . .	$0{\cdot}2486/[2{\cdot}6617 - \log (458{\cdot}9 - V)]$
Holde . .	$0{\cdot}4470/[2{\cdot}1920 - \log (155{\cdot}6 - V)]$

The conversion of empirical into absolute values of viscosity has been the subject of quite a large amount of work, and many new conversion factors have become available.

Herschel (*Chem. Met. Eng.*, 1922, **26**, 1175) has provided conversion tables applicable to the Saybolt "Furol," and "Universal" and Redwood No. 2 viscometers. The Saybolt "Furol" viscometer is useful for bunker fuels for which the "Universal" is unsuited. The dimensions are the same, except for the diameters (internal and external) of the outlet tubes. The "Furol" time in seconds, t, is convertible into absolute units according to the formula $0{\cdot}022t - \frac{2{\cdot}03}{t}$; the Redwood time is convertible by the formula $0{\cdot}0293t - \frac{0{\cdot}403}{t}$.

Fortsch and Wilson (*Ind. Eng. Chem.*, 1925, **17**, 291) have also defined Saybolt units in terms of absolute units by calibrating the viscometer by means of liquids of known viscosity. Errors are introduced, owing to differences in capillary rise due to surface tension, although the rise in surface tension from light petroleums to light lubricating oils is only slight. Means are suggested to render the results trustworthy for all liquids, irrespective of capillary rise.

Formula for the Ostwald Viscometer.—Vogel (*Z. angew. Chem.*, 1922, **35**, 561) has modified the Ostwald viscometer in such a way that his results give the ratio of the viscosity in centipoises to the specific gravity of the liquid. By means of chemically pure substances, he has established a formula for reducing Engler type readings to absolute viscosities. The results agree well with liquids ranging in specific gravity from 0·6 to 1·5. The viscosity-temperature curve is calculated from the formula $\eta t = \eta_\infty (t - t_1)(t - t_\infty)$, t_1 being the temperature at which the oil has the fluidity of water at 20·2°, and t_∞ that at which the viscosity is infinite and the fluidity zero. He adds that the shape of this curve is more important than a numerical value at any one temperature. This observation was confirmed by König (*Z. angew. Chem.*, 1924, **37**, 8), who states, however, that the equations of Vogel, of Schwedhelm (*Chem. Ztg.*, 1920, **44**, 638; 1921, **45**, 41) and of Van Aubel (*Compt. rend.*, 1921, **173**, 384) contain constants which cannot be determined with sufficient accuracy. One means of overcoming the difficulty is to differentiate Vogel's equation:

$$d\eta/dt = \frac{100a^2}{0{\cdot}4343 \times 2(t - t_\infty)^2} \text{ per cent.}$$

New Methods of Viscometry.—In addition to improvements of older methods, several new methods are of special interest and value.

Don (*Kolloid Z.*, 1924, **34**, 312), remarking that the lubricating power of an oil is affected by two factors—adhesion, chiefly measured in Ostwald's viscometer, and cohesion, chiefly measured in Redwood's viscometer—has devised a method intended to cover both of these factors. A cylinder is packed with small steel

spheres to expose a large surface. The head of oil above the cylinder is kept constant while the weight of a given number of drops and their rate of fall is determined below. The results show the combined effects of viscosity and surface tension.

Borodulin (*Petroleum*, 1925, **21**, 445) has devised a simple method for determining the viscosity of oils. A glass jet delivers 27 drops of water per c.c. One such drop is timed over a fall through 10 cm. of oil, maintained at a constant temperature. The result is roughly proportional to the Engler time.

Berl, Isler and Lange (*Z. angew. Chem.*, 1924, **37**, 128) have devised a method for determining the viscosities of very viscous substances. It involves no more than simple modifications to a laboratory balance, and the determination of the resistance to oscillation of the balance exerted by virtue of a wire dipping into the oil. The results agree with Engler times for heavy oils, although not for light oils. The method is especially suitable for oils containing suspended matter, if this is not excessive.

An equally simple method is due to von Dallwitz-Wegner (*Petroleum*, 1926, **22**, 1048). A rotating worm in a cylinder of oil in a vessel in a constant temperature bath causes the oil, by virtue of its centrifugal force, to rise in a vertical tube which is graduated in any suitable units.

Herbst (*Chem. Ztg.*, 1929, **53**, 344) has devised an apparatus by means of which, with suitable adjustments, viscosity, lubricating power of metal surfaces and angle of contact can be measured; even pitches and resins may be tested by this method.

As viscosity is not an additive property, Espy (*Petroleum* (Chicago), 1919, **8**, 27) has evolved an empirical chart to determine the viscosity of a mixture of two oils.

A law has also been evolved experimentally by Leu (*Monit. Pétrole Roumain.* 1923, (23–24), pp. 16) for calculating the viscosities of mixtures of mineral oils. It takes the form—

$$\frac{\mathrm{P}}{p}\ \frac{(a_1 - a)(a_1 + a_2)}{2(a - a_2)(a_1 - a_2)} = e^r$$

where a_1 and a_2 are the constants for the constituents, and a that for the mixture constituted in the proportions p and P, respec-

tively, percentages by volume. For mineral oils $x = 1$, whence

$$\frac{P}{p} = \frac{(V - V_1)(V + v)}{2(V_1 - v)(V - v)} \quad (= 2{\cdot}71828).$$

V and v are the Engler viscosities of the constituents occurring in the proportions P and p respectively, and V_1 is the viscosity of the mixture.

The most recent work on the temperature coefficient of viscosity has been carried out by Dean and Davis (*Chem. Met. Eng.*, 1929, **36**, 618). They have determined the variation of the Saybolt viscosity with temperature over the range 100° to 210° F. If the Saybolt viscosity at 100° F. is Y and at 210° is x,

$$Y = a + bx + cx^2,$$

where a, b and c are characteristic for different oils; from these constants was compiled a series of "viscosity indices": the viscosity index is zero if the temperature coefficient of viscosity is large, and is 100 if the temperature coefficient is small.

Relationship of Viscosity to Composition.—Two investigations have linked viscosity determinations with the composition of the oil and appear well worthy of further development. Hill and Ferris (*Ind. Eng. Chem.*, 1925, **17**, 1250) have studied the variation of viscosity, and of the temperature coefficient of viscosity, with the boiling point (as given by the 50 per cent. point on the vacuum assay distillation test, *q.v.*), and have found for fractions of the same boiling point an increase as the nature of the oil passes from paraffinic to naphthenic. This variation should consequently be indicative of the source of the oil.

Hill and Coats (*Ind. Eng. Chem.*, 1928, **20**, 641) have observed, between the Saybolt viscosity and specific gravity, a mathematical relation which is a constant value calculable for any oil and is indicative of its chemical composition, being higher for naphthenic and lower for paraffinic products. Also, if it is higher, the viscosity has a higher temperature coefficient. The relation holds for distillates of narrow boiling range and for blends of widely different viscosities, although fractions from mixed crude oil may give variable results.

Flash Point.—While the flash point of an oil retains its signifi-

cance as a measure of the properties of volatility and inflammability combined, it has not advanced materially as a means of evaluating oils; in this respect the spontaneous (or auto-) ignition temperature is of greater significance. Practically no changes have been made in either the methods or apparatus employed.

Schluter (*Chem. Ztg.*, 1928, **52**, 261) has suggested altering the position of the thermometer in the open flash point test, but Friedbach (*Petroleum*, 1929, **25**, 93) points out that this is unnecessary, as the possible variations are not greater than experimental error.

More important factors are the rate of heating, and the position and length of the ignition flame, with avoidance of local heating. It is even suggested that some methods of flash-point determination are too sensitive: Terpugoff (*Petroleum*, 1929, **25**, 1161), referring to the Pensky-Marten apparatus, states that differences of many degrees are often observed in oils of the same commercial quality.

Thiele (*Ind. Eng. Chem.*, 1927, **19**, 259) has observed that, in the case of blended oils, the antilogarithm of one-hundredth of the flash point is an additive property. Assuming that the flash point is the temperature at which the vapour pressure reaches 10 mm. of mercury, he has verified his observation, except in the case of oils, such as kerosene, of low flash point.

The Spontaneous Ignition Temperature.—The spontaneous ignition temperature of liquid fuels is a property which has lately excited much attention. Moore's well-known ignition meter (*J. Soc. Chem. Ind.*, 1917, **36**, 109) has been the subject of much experiment by Ormandy and Craven (*J. Inst. Pet. Tech.*, 1924, **10**, 335; 1926, **12**, 650), who have found that careful purification of the oxygen (*i.e.*, removal of all traces of hydrogen) effects a rise in the ignition temperature (*e.g.*, of heptane from 245° to 253°). They consider their determinations to be accurate to 1°, but add that very slight alterations in the experimental conditions have an appreciable effect. Ionising and drying the oxygen have no effect, but the substitution of the vapour of the liquid for the liquid itself causes a rise of 5°. The spontaneous ignition temperatures of a series of compounds are given and their significance discussed.

Moore himself (*J. Inst. Pet. Tech.*, 1920, **6**, 186) has recorded that there is no substantial alteration in the ignition value by varying the rate of supply of fuel, or of air or oxygen; by diluting with carbon dioxide; or by using crucibles of different materials (silica, platinum, nickel, porcelain). Bridgeman and Marvin (*Ind. Eng. Chem.*, 1928, **20**, 1219), on the other hand, have claimed that the ignition temperature depends upon the material and volume of the vessel, the concentration of oxygen, the pressure in the system, the composition of the fuel, and the time lag. As the result of experiments carried out in a bomb similar to the combustion chamber of a Liberty engine, they have found that the time to produce firing depends upon the rate of evolution of heat by combustion and the rate of heat exchange between the charge and the containing vessel, so that ignition temperatures which involve a time lag are composite functions of properties of the fuel and the apparatus. Firing usually occurs when the temperature of the charge is raised by chemical action above that of the container; this is the *auto-ignition temperature*, and is of interest in considering fire risks. If there is no time lag, the *true ignition point* is obtained, which is a fundamental property of the fuel, given definite pressure and concentration conditions. It is suggested that the latter might best be measured by heating the combustible mixture by means of an ideal adiabatic compression, but experimental difficulties would involve an extrapolation to obtain this point.

Thompson (*Ind. Eng. Chem.*, 1929, **21**, 134) has investigated the effect of the nature of the vessel used in ignition-point determinations. With a copper vessel heating is more regular, but the results are unreliable, owing to the subsidiary action of the reduction of copper oxide; chromium-plated copper and steel are better, but still unsatisfactory. Changes in shape and in quality of the glass vessel have little effect.

Jentzsch (*Z. Ver. D. Ing.*, 1924, **68**, 1150) has devised an "ignition value" obtained by dividing the spontaneous ignition temperature by the number of bubbles of oxygen passing per minute. This ignition value would not seem to be a property of any great significance; it is a function not only of the fuel, but

also of the temperature. The work has, however, led to the patenting of a special apparatus (Eng. Pat. 230,484, March 7th, 1925).

Volatility.—The volatility of petroleum oils is of special interest only in the case of oils used in internal combustion engines. In such oils an indication is given by a study of the boiling-point curve, but this indication is not definite enough for modern requirements. For easy starting the oil should contain sufficient of the low-boiling fraction, and for complete combustion it should not contain too high a proportion of high-boiling fractions. The question is complicated also by such questions as whether the latent heat of vaporisation is high or not, since a high latent heat will counteract the advantage of a low boiling range.

"*Dew Point.*"—The boiling-point curve, considered as satisfactory in 1920, has, therefore, been supplemented by other tests which have a more direct bearing upon engine performance. For the determination of "dew-point" several methods have been proposed. Wilson and Barnard (*J. Ind. Eng. Chem.*, 1921, **13**, 906) have calculated the initial condensation temperature of petrols from the vapour pressure of the original material and that of the equilibrium boiling mixture. The results suggest that failure to vaporise petrol completely is due to defects in carburation rather than to shortcomings in the petrol itself. A method which gives somewhat higher values is due to Gruse (*J. Ind. Eng. Chem.*, 1923, **15**, 796), who passes the spirit through a needle valve on to a heated gauze and thence into a chamber containing a metal mirror. The temperature of the chamber is lowered until the dimming of a light, reflected in the mirror, gives the dew-point. A third method is due to Stevenson and Babor (*Ind. Eng. Chem.*, 1927, **19**, 1361), who vaporise the spirit in a jacketed tube and cool the tube at a suitable rate till the dew-point is reached.

An elaboration of dew-point measurement is the drawing of a graph for the dew-points of all fractions up to 100 per cent. evaporation.

"*Evaporation Number.*"—A purely evaporative method has been used by Polcich and Fritz (*Brennstoff-Chem.*, 1924, **5**, 371). Ten c.c. of spirit are placed in a special vessel and evaporated in a stream of air, passing at the rate of 6 litres per minute. The

volumes of air necessary to evaporate 9·5 and 10 c.c. are noted, the latter being termed the "evaporation number." The percentage of a motor spirit which is evaporated in practice is difficult to determine; Gramenicki has shown that it depends upon a variety of factors, such as temperature, pressure, volume, surface and time, and has suggested a means of measuring the percentage.

Measurement of Vapour Pressure.—A different means of evaluating the "starting" properties of a fuel is by measurement of its vapour pressure. Tizard and Marshall (*J. Inst. Pet. Tech.*, 1922, **8**, 217) have evolved the following procedure:

Into the top of a 100-c.c. reservoir are sealed a tap and a burette, and to the lower end are attached a U-tube and manometer. Mercury is admitted to the burette up to a certain mark and dry petrol introduced. The pressure is reduced below atmospheric, and the temperature to 0°. The pressure obtaining, $p_1 = \frac{p + (P_o V + 760\,vx)}{V - v + yv - P_o}$; where P_o represents the original pressure of the air; p, the true vapour pressure of the liquid; V, the volume of the reservoir; v, the volume of the liquid; x, the amount of dissolved air; and y, the air necessary to saturate the liquid. If V is very small, $p_1 = p$. The amount of dissolved air in petrol is 0·22 volume, and in benzene 0·15 volume.

Rhodes and McConnell (*J. Ind. Eng. Chem.*, 1923, **15**, 1273) have used the very simple method of measuring the vapour pressure in a glass globe in a thermostat after expelling the air by boiling the spirit. No relation was observed between the pressure-temperature curve and the specific gravity or average distillation temperature.

Davis (*Ind. Eng. Chem.*, 1925, **17**, 1136) also has used a simple method. The pressure (A) of a mixture of petrol vapour and saturated air is measured, the mixture compressed to half (pressure B) and to one-third of its volume, and the pressures measured. The vapour pressure of the spirit = P = 2A − B. The error due to the presence of dissolved air in the spirit is ascertained by finding how much higher the calculated value of P is at one-third V. The method is a modification of the U.S. Bureau of Explosives bomb method.

Wilson (*Ind. Eng. Chem.*, 1928, **20**, 1363) has constructed a nomogram for calculating the vapour pressure of hydrocarbon

mixtures. By means of an empirical equation, he obtains the vapour pressures of the normal hydrocarbons in terms of temperature and boiling point at atmospheric pressure. The application of the chart is limited, because paraffins other than the normal ones occur, and even close fractionation does not effect a good separation.

Refractometry of Petroleum Products.—Utz (*Petroleum*, 1921, **17**, 1295) has reviewed the work on the refractometric examination of petroleum products. He states that the refractive index increases with boiling point or melting point, but that it is a property which serves little purpose except that of distinguishing certain American and European crude oils. It has been used, to some extent, to detect the admixture of other substances. Eckart (*Brennstoff-Chem.*, 1923, **4**, 24) has examined petrols, benzols, ethyl alcohol and tetralin (with a Zeiss-Pulfrich refractometer, using the sodium D lines). For petrols the refractive index increases with the specific gravity; the other substances have a fairly constant value.

Hoyte (*J. Inst. Pet. Tech.*, 1925, **11**, 76) has examined motor fuels with regard to their content of aromatic hydrocarbons; for benzene, toluene and xylene the index varies from 1·4985 to 1·5082; for petrols it is not greater than 1·43. If the aromatic hydrocarbons are removed from a spirit of known refractive index and then a mixture of benzene and toluene in equal proportions is added in quantity sufficient to give the original result, an estimate of the aromatic hydrocarbon content is obtained. It was also found that the refractive index curve for heptane-toluene mixtures is nearly rectilinear. Dietrich (*Chem. Ztg.*, 1927, **51**, 509) has examined in a similar way petrol-benzene mixtures with an immersion instrument, and confirms the conclusion that petrol has the lowest gravity and that the refractive index affords a convenient means of detecting the presence of aromatic hydrocarbons. The precautions to be observed in such a process are enumerated.

Colour Testing of Lubricating Oils.—The importance of colour in testing lubricating oils is not great (W. H. Herschel, *Proc. Eng. Soc. W. Pa.*, 1923, **38**, 503). The colour scales agreed upon by the

Institution of Petroleum Technologists and the American Society for Testing Materials are those of the Saybolt, Stammer and Lovibond colorimeters.

Parsons and Wilson (*J. Ind. Eng. Chem.*, 1922, **14**, 269), during an investigation of the colours of blends of oils, found that the Lovibond tintometer did not show the arithmetic mean of a blend. This suggests that the Lovibond scale is not a rectilinear representation of increase in intensity of colour. A blend of kerosene and dark oil was made to correspond with Lovibond No. 50. An oil under examination was then compared with this standard by varying the depths of the two oils, monochromatic light being used, involving the use of Beer's law (which was confirmed experimentally). Thus a true scale was obtained and was plotted against the Lovibond scale. This affords a means of predicting the Lovibond number of a blend and is a factor in research on decolorising agents.

Grunewald (*Petroleum*, 1927, **23**, 1621) has constructed a similar colour scale of twenty-six degrees by mixing "water-white" kerosene and heavy oil in standard vessels. Romberg (*Petroleum*, 1922, **18**, 361) has devised a formula for calculating the Stammer colour value of a mixture: its reciprocal takes the form $A/C_A + B/C_B + \ldots$ where A, B . . . are the fractional values of the respective constituents and C_A, C_B . . . the corresponding Stammer colour values. The Stammer method was applied to wax by comparing the light reflected from a planed wax surface, 1 cm. thick, with the colour of standard potassium dichromate. The method was used to compare the relative efficiencies of a series of bleaching agents.

Surface Tension and Emulsification. Gurwitsch (*Petroleum*, 1922, **18**, 1269) has observed that surface tension is an important property in the consideration of the suitability of oils for turbine lubrication. A tendency to emulsify is undesirable, and, as the presence of asphalts lends persistence to films and promotes the formation of stable emulsions, they, together with the surface tension, should receive consideration. For marine engines, steam cylinders and cutting oils, however, emulsification is a property to be desired. It is promoted by petroleum soaps, naphthenic

acids and fine sand or mud, and emulsions are resolved by alcohol.

Francis and Bennett (*J. Ind. Eng. Chem.*, 1922, **14**, 626) have observed that surface tension tends to increase with specific gravity, but is apparently unaffected by the presence of fatty acids and waxes. For petroleum fractions generally, the temperature coefficient of surface tension is 0·05 dyne per sq. cm. per degree Fahrenheit. The increase of surface tension with viscosity is very small; thus, for oils ranging in viscosity from 51 to 1,160 seconds (Saybolt), the surface tension was found to increase only from 34·4 to 37·3 dynes per sq. cm.

Harvey (*Ind. Eng. Chem.*, 1925, **17**, 85) has used the du Noüy tensiometer (*J. Gen. Physiol.*, 1919, **1**, 521) for petroleum oils, and finds that it gives slightly higher results than the capillary tube and drop methods.

Doi's apparatus (*loc. cit.*, "Viscosity") for determining the combined effect of viscosity and surface tension, has been described.

MISCELLANEOUS TESTS

Consistence of Lubricating Greases.—Penetration methods have proved to be most suitable for determining the consistence of greases.

Karns and Maag (*J. Ind. Eng. Chem.*, 1923, **25**, 716) measure the depth to which a falling ball penetrates. If the grease is hard the ball is allowed to fall on to a pin, the penetration of which is measured. Normann (*Chem. Umschau*, 1925, **32**, 115) uses a carefully standardised method in which a brass rod of weight 50 grms., 20 cm. long by 9 mm. diameter, with a coned end 9 mm. long, is dropped into the grease contained in a cylindrical box, 14 cm. high by 16 cm. in diameter. The rod is engraved with a millimetre scale.

Softening Point.—The rather indeterminate nature of the softening point of waxes, resins, asphalts and pitches has constantly evoked new methods for obtaining reproducible results.

Nashan's "malakograph" (*Chem. Ztg.*, 1922, **46**, 386) consists of a balance beam, at one end of which is suspended a sphere,

whilst the other end carries a heavier weight with a pen, which makes a record on a chart fastened to a revolving drum. The sphere rests in a vessel filled with the wax, which is melted and then allowed to re-solidify, so that it grips the sphere. The wax is then re-heated slowly; at first the pen describes a straight line, but as the wax softens this becomes a curve, and the temperature corresponding to the transformation of the straight line into the curve is taken as the softening point.

An apparatus very similar in principle is due to Herbst (*Chem. Ztg.*, 1927, **51**, 140; *Petroleum*, 1927, **23**, 1079). A brass rod and a weight are suspended over a pulley. The brass rod is heavier than the weight, and it rests on the surface of the wax in a suitable container. The pulley shaft carries a pointer which records on a dial the sinking of the brass rod into the wax as the wax is raised in temperature.

Oil in Crude Wax.—The view is quite generally held that the "press method" of determining oil in wax by extrusion (with the use of cloth or filter paper) is unsatisfactory, especially if the amount of oil exceeds 10 per cent. Wyant and Marsh (*U.S. Bur. Mines Tech. Paper* 368, 1925, pp. 26) prefer to use an extraction method, with acetone as the solvent. Henderson and Ferris (*Ind. Eng. Chem.*, 1927, **19**, 262) have modified this method to use nitrobenzene as the solvent. The wax (10 grms.) is weighed into a flask with twice its weight of nitrobenzene, air is bubbled through, and the temperature is raised to 70°, and is then reduced to about 30°. The wax solidifies and floats; the oil is withdrawn, more nitrobenzene is added, and the temperature again raised to 135° to 150°. The yield of wax and its melting point thus obtained agree with those obtained from sweating ovens.

A refractometric method is described by Diggs and Buchler (*Ind. Eng. Chem.*, 1927, **19**, 125). The amount is calculated, with certain provisos, from the refractive indices of oil-free wax and wax-free oil. If the amount of oil is small, this method gives higher results than the press method. A method which is probably superior is due to Wilson and Wilkin (*J. Ind. Eng. Chem.*, 1924, **16**, 9). About 5 grms. of wax are dissolved in 75 c.c. of ethylene dichloride, the solution is cooled to −18° and filtered,

and the wax is washed with a further 75 c.c. of the solvent. The filtrate is evaporated, and the oil dissolved in a mixture of mineral, seal and ligature oils made up to give the same refractive index as the wax. From a curve constructed from mixtures of known composition the refractive index gives the amount of oil. The method gives concordant results and shows 0·1 to 0·3 per cent. of oil in refined wax, and 2 per cent. in crude wax.

Specific Gravity of Paraffin Wax.—Morris and Adkins (*Ind. Eng. Chem.*, 1927, **19**, 301) took special precautions for excluding all air bubbles from a sample of paraffin wax and have determined its specific gravity by means of a glass Nicholson's hydrometer of special construction. The results at 15·5 differ greatly from those calculated by observations at higher temperatures. The curve obtained by plotting specific gravity against temperature shows a break at the melting point, and also shows other "flat spots" of unknown significance.

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CHAPTER X

COAL

By J. G. King, Ph.D., F.I.C., and R. A. Acton Taylor, M.Sc., F.I.C.

Position in 1922—General Progress—Proximate Analysis—Calorific Value—Agglutinating Value—Carbonisation Assay Methods—Coal Ash—Coal Constituents—Rational Analysis—Pulverised Fuel.

Some eight years ago the evaluation of coal by means of analysis had progressed to a very slight extent in comparison with the great industrial importance of coal and its products.

The common test, proximate analysis, was subject to wide variations in technique and to correspondingly great discrepancies between different laboratories or even observers. Recognition of this had led to the laying down of certain standards for moisture determinations by the International Congress of Applied Chemistry (*Eighth Congress*, 1912, **25**, 41) and of complete methods by a joint committee of the American Chemical Society and the American Society for Testing Materials (adopted 1916, 1918; revised every three years). In this country standardisation was first attempted by a committee of the Fuel Research Board, but their first report was not published until 1923.

Despite this standardisation, it appears certain that the common methods of analysis employed in industry were by no means reliable. Since then, however, the spread of scientific work in the coal industry has done much to eradicate rule-of-thumb methods and establish those approved by the above committees.

The determination of the elementary composition of the coal was carried out to a much smaller extent than the proximate analysis. The method employed for the determination of carbon and hydrogen was the well-known one of Liebig and Dennstedt

of combustion over copper oxide or platinum. Reference had then been made to the importance of correcting the value for carbon by determining the amount present as carbonate (Sinnatt and Harrison, *Lancashire and Cheshire Coal Research Assn. Bull.*, No. 7, 1920), but the correction had not been widely applied. Similarly, although a method for the separation of the total sulphur as pyritic, organic and sulphate sulphur had been put forward by Powell and Parr (*Univ. Ill. Eng. Expt. Sta. Bull.*, 111, 1919), very little use had been made of it. For the determination of nitrogen both the Kjeldahl and Dumas methods were in use, but the latter was already fast disappearing.

The only other element considered to be important was phosphorus, and the most favoured method for its determination involved solution of the ash and precipitation of the phosphorus by the molybdate method.

Recognition of the fact that proximate and ultimate analyses gave only very incomplete information as to the industrial value of a coal had led to the evolution of certain empirical methods designed to give supplementary information, particularly with reference to the evaluation of gas and coking coals. The Campredon test for agglutinating value (*Compt. rend.*, 1895, **121**, 820) had become the most commonly applied, but the difficulty of obtaining a standard sand was a bar to its extensive use. Sinnatt and Grounds (*J. Soc. Chem. Ind.*, 1920, **39**, 83T) had recommended electrode carbon as an alternative diluent which gave considerable promise.

Other methods of evaluating coking coals, involving the separation of the coal into fractions by solvent extraction, were exciting considerable interest, but at this time were still considered as research methods.

Methods involving small-scale carbonisation were beginning to attract attention on the Continent, the method of Bauer (original thesis, 1908; see Schramm, *Gas u. Wasserf.*, 1913, **56**, 389), involving the distillation of 15 grms. of coal and the passing of the products over a cracking zone, had been designed to simulate the conditions of high-temperature carbonisation and to evaluate coals for coke and gas manufacture. In this country Lessing

(*J. Soc. Chem. Ind.*, 1912, **31**, 465, 671) had designed a simple apparatus, operating on 1 grm. of coal, for the evaluation of the coking properties of coal and its value as a gas coal. Real attention was not, however, directed to such methods until after 1923.

Recognition of the fact that a calorimeter of the bomb type was the only satisfactory instrument for the accurate determination of the calorific value of coal had become fairly widely known, but in commercial laboratories its use was by no means extensive. A valuable paper by Huntly had given a clear statement of the accuracy obtainable with such a calorimeter (*J. Soc. Chem. Ind.*, 1910, **29**, 917).

General Progress.—In those methods of analysis which are in general use there has been very little change in the last eight years. The work of standardisation committees has helped to smooth out irregularities and to reduce the number of variables. The most important of these committees are the American Chemical Society and A.S.T.M. and that of the Fuel Research Board. The former have prepared every third year from 1918 a revised edition, and their methods have been closely followed in America. In this country the Fuel Research Committee have followed up an interim report, published in 1923, with a final statement, published in 1927 (*Fuel Research Survey Paper*, 7, 1927). In addition, methods have been issued by the British Engineering Standards Association, both for the export trade and for industrial use. There still remain, however, some methods the present form of which is not fully approved.

PROXIMATE ANALYSIS

The methods laid down by the Fuel Research Committee (1927) still remain as accepted standards.

Moisture. The most favoured method for the determination of moisture in coal is that in which 1 grm. of the coal is dried at about 105° for one hour in an inert atmosphere. The most suitable vessels appear to be shallow silica dishes with a ground lip and a ground-on cover. After the moisture has been determined it is

possible with such dishes to proceed to the ash determination with the same sample and dish. New methods employing extraction of the water with solvents have been suggested in cases where speed is important. That put forward by Mannheimer (*Ind. Eng. Chem.* (*Anal.*), 1929, **1**, 154) would appear to be sound. The coal is extracted with methyl alcohol, and the density of the diluted alcohol is determined indirectly by observing the equilibrium temperature for a floater, and this equilibrium temperature, by reference to a chart, is interpretable in terms of the moisture content of the alcohol. A similar, but less straightforward, method is suggested by Doleh (*Proc. 2nd Int. Conf. Bit. Coal*, 1928, **2**, 425).

Ash. - The burning of the combustible material and the weighing of the residual ash is a process which has never been altered or amplified appreciably. A method of dealing with the errors due to the loss of water of hydration of silicates and to the conversion of carbonates to oxides and pyrites to ferric oxide is mentioned under errors in elementary analysis.

Volatile Matter. The empirical nature of this test has led to the fixing of strict conditions, and these should not be departed from. Experimental work has shown that if an air-free muffle furnace is used, the platinum crucible may be replaced by silica or porcelain.

The Lessing carbonisation assay apparatus (*q.v.*) has also been proposed for the determination of volatile matter.

In determining the volatile matter in coke, combustion of the sample inevitably causes serious discrepancies if the crucible method is used. A Fuel Research method is recommended in which the coke sample is heated in a slow stream of nitrogen. Details of this method have not yet been published.

ELEMENTARY ANALYSIS

The "ultimate" analysis of coal has now come to mean the determination of the elements: carbon, hydrogen, nitrogen, sulphur, phosphorus and arsenic, with the supplementary determination of carbon as carbonate, and the differentiation of the

sulphur as sulphate, pyritic and organic sulphur. In addition, a distinction is now drawn between the sulphur which is driven off on combustion and that retained in the coal ash.

Carbon and Hydrogen.—Practically no change has been made in the technique of the combustion methods, but the Liebig method, employing copper oxide, has become the most popular. King and MacDougall (*Fuel*, 1926, **5**, 33) have shown that, under the conditions of the Fuel Research Standard methods, the important consideration is the temperature of the copper oxide. If the oxide is retained at a temperature of 800°, the method becomes relatively insensitive to variations in the rate of combustion, and, even with very rapid heating, the error in the carbon percentage should be less than 0·15 per cent.

The use of the calorimetric bomb for the determination of carbon has been suggested (Goutal, *Fuel*, 1923, **2**, 344), the gaseous products of the combustion being passed through a drying train and the carbon dioxide absorbed in the usual manner. The method is, however, somewhat laborious, and is not likely to be widely applied (see also Watkins, *Ind. Eng. Chem.*, 1927, **19**, 1052, and Carr and Rente, *Ibid.*, 1928, **20**, 548).

Nitrogen.—The Kjeldahl method for the determination of nitrogen consists simply in the digestion of 1 grm. of the coal with 30 c.c. of 96 per cent. sulphuric acid to which has been added 10 grms. of potassium bisulphate and 1 grm. of mercury. This method, put forward by the Fuel Research Committee, has been considered by Baranov and Mott (*Fuel*, 1924, **3**, 31, 49) in comparison with other modifications and methods, and is thought to be the most reliable.

The Dumas method is still favoured in certain laboratories, but it is now recognised that the gas collected in the nitrometer should be analysed and the result corrected for insoluble gases other than nitrogen.

Sulphur. Many comparisons have been made during the past few years of methods for the determination of sulphur (*e.g.*, Selvig and Fieldner, *Ind. Eng. Chem.*, 1927, **19**, 729). For coals containing less than 2 per cent. of sulphur the consensus of opinion is that the Eschka method gives the most accurate result

(*cf.* Forster and Probst, *Brennstoff-Chem.*, 1923, **4**, 357). The use of a special combustion bomb is gaining in favour, however, and has much to recommend it. Using a calorimetric bomb, King and Crossley (*Fuel*, 1929, **8**, 544) have shown that an accuracy of ± 0·2 per cent. is possible if the sulphur in the ash is brought into solution by fusion, and that the error occasioned by absorption of sulphur in the ash is not greater than 0·1 per cent.

Errors Occasioned by Inorganic Matter.—Tideswell and Wheeler (*Amer. Inst. Min. Met. Eng. Tech. Pap.*, No. 104, 1928) have devised means for correcting certain errors in the ultimate analysis of coal. When a coal is ashed, the ash figure is low by an amount representing the loss of water of hydration of silicates and the conversion of carbonates to oxides, and pyrites to ferric oxide. The first of these sources of error is not accounted for in the moisture determination, but it gives a wrong value to the percentage of hydrogen; the second gives a wrong value to the carbon. Furthermore, the algebraic sum of all these errors is reflected in that difference figure which is taken to mean the oxygen content, and the calculation of the ultimate analysis to the ash-free basis is also rendered erroneous.

Phosphorus.—It has become generally recognised that the molybdate method (*Fuel Research Survey Pap.*, No. 7, 1927) is the most satisfactory for the determination of phosphorus in coal ash, and it is now being widely used. Doubts exist, nevertheless, as to whether it is really accurate, as other elements, such as titanium, interfere. A relatively simple method of treatment has been evolved by Skilling and Ballantyne (*J. Soc. Chem. Ind.*, 1929, **48**, 115T), in which the phosphorus is brought into solution by the use of sulphuric acid, prior to determination by the molybdate method. This method has given very satisfactory results on synthetic mixtures containing titanium and is worthy of wider application and trial.

The finding of a reliable method for the determination of phosphorus is now becoming a matter of some urgency in the iron and steel industries.

Arsenic.—The Marsh test was the first to be applied for the

quantitative determination of arsenic in coal, but the difficulty in this method is the preservation of standards of comparison. Further research work will be necessary before a satisfactory method is found. In the meantime the method which shows greatest promise is that of Gutzeit (Lunge and Keane, Vol. I., Pt. I., 374), as the electrolytic method of Thorpe (*J. Chem. Soc.*, 1903, **83**, 974) is rather expensive.

CALORIFIC VALUE

In recent years the bomb calorimeter has been gradually ousting the less accurate types. The introduction of stainless steel has helped by allowing reductions in price, and the replacement of the troublesome lead washer by a rubber one requiring little more than hand pressure has simplified manipulation.

The adiabatic calorimeter introduced by Richards (Harvard, 1906) will give greater accuracy than the common type equipped with a constant-temperature water-jacket, but for short experiments, such as determinations of calorific value, the advantage is small if certain precautions are taken. White and others (White, *The Modern Calorimeter*) have shown that the chief sources of error lie in (1) temperature measurement, (2) ineffective stirring, (3) evaporation losses, (4) convection currents, and (5) the difficulty of following a rapid rise of temperature.

The errors in temperature measurement can be overcome in very exact work by the use of groups of thermo-couples. For normal work it is possible that the solid-stem variable-range thermometers now available will give better results than the enclosed-scale Beckmann type.

Errors due to evaporation can be overcome by the use of tight covers, and convection currents should be preventable by the introduction of baffles in the form of convection shields.

To overcome errors such as the above, Landrieu (*Bull. Soc. Chim.*, 1925, **37**, 1340) has suggested the use of sealed containers for the water, and recommends always starting determinations at the same temperature. He finds that the stirrer effect is proportional to the cube of the velocity.

AGGLUTINATING VALUE

The difficulty of obtaining concordant results with the Campredon "caking index" test has led to the suggestion of several improvements and alternatives. The chief difficulty was that of finding a suitable sand, and although concordant results could be obtained with suitable sand (Gray, *Fuel*, 1923, **2**, 42), sands from other sources did not give the same results. Electrode carbon, suggested by Sinnatt and Grounds (*J. Soc. Chem. Ind.*, 1920, **39**, 83T), was a good alternative, but, unfortunately, the suggestion does not seem to have been followed up.

On the Continent the somewhat similar Meurice test (*Ann. Mines Belg.*, 1914, **19**, 625) is more generally in use.

A modification of the Campredon test has been suggested by Barash (*Fuel*, 1927, **6**, 532). The coal is carbonised in admixture with electrode carbon, the proportion being varied until the coke obtained is just non-coherent. Barash has shown that the proportion of carbon to coal, his "agglutinating value," is reproducible, and is therefore a characteristic property of the coal.

A new method, which appears to be promising, is that of Marshall and Bird (*Amer. Inst. Min. Met. Eng., Tech.* 216). The coal is mixed with a special sand, consisting only of round grains, compressed into a briquette, the briquette carbonised, and its strength measured in a special apparatus. The method is really an elaborated form of Kattwinkel's test (*Gas u. Wasserf.*, 1926, **69**, 145), and, although it has not yet been applied to more than a limited extent, it appears to bear considerable promise of success.

At the present time comparatively little faith is being placed in agglutinating values, and it is most desirable that one or other of the methods should be closely investigated and placed upon a sufficiently sound basis to allow of its general use. In the meantime more reliable information is obtained by examining the coke from a carbonisation assay conducted after the manner described in *Fuel Research Tech. Paper*, 21, 1929, 16–17.

Analysis by Carbonisation Assay Methods. The examination of coal by the carbonisation of small quantities in the laboratory has been developed mainly with the idea of correlating the type

of coke formed and the yields of products with those obtained in carbonisation processes. The best known are those of Lessing and of Bauer, and in some form these are used widely for the control of coal used in coke ovens and in gas manufacture. In its most elaborate form the Bauer method is widely used on the Continent (Litinsky, *Die Messtechnik*, 1926, Nov.; *Gas J.*, 1926, **176**, 502), while a very similar method is in use in America (*U.S. Steel Corporation Methods*, p. 130).

A second type of assay method which has been evolved is more strictly analytical in its aims. The Lessing method falls into this class also, in that it can be used for the measurement of the swelling power of coals on carbonisation. The Gray-King assay (*Fuel Res. Tech. Pap.*, No. 1, 1921; No. 21, 1929) has been so arranged as to yield information which is supplementary to that of analysis, and which allows of more precise differentiation between coals than is possible by analysis alone. For this reason the method is being employed in the Fuel Research Survey of National Coal Resources. The method allows for the determination of yields of coke, tar, gas and liquor on a 20-grms. scale, and, by the application of easily determinable conversion factors, these may be interpreted as an approximation to the behaviour of the coal in a carbonisation plant. The nature of the coke which the coal will yield is also clearly shown. A modification of the method permits of its use as an industrial process assay of the first type for the evaluation of gas coals (*Fuel Res. Tech. Pap.*, No. 24, 1930).

COAL ASH

The importance of the inorganic constituents in coal has increased steadily, notably in relation to coal cleaning, but also in connection with the problem of dust deposition in the neighbourhood of pulverised fuel plants.

A suggestion was made in 1898 by Couriot (*Bull. Soc. Ind. Min.*, 1898, **12**, 713) that the distribution of the inorganic matter in coal could be observed in coal by examination under the X-rays. Other workers, notably Kemp (*Proc. Inst. Min. Eng.*, 1924, **67**, 59), have followed up this work and have endeavoured to make the

observations quantitative. Up to the present, however, the variable nature of the inorganic matter has proved a difficulty, and the value of the method lies only in its qualitative, or comparative, application.

Fusion Temperature.—Methods for the determination of the fusion temperature of coal ash have become more commonly applied, partly owing to the growth of pulverised fuel firing.

The U.S. Bureau of Mines method, described by Fieldner, Hall and Field (*Bull.*, 129, 1918), has become widely applied in America. In this country an essentially similar method has been standardised by the Fuel Research Division upon duplicate samples of ash (King, Blackie and Millott, *Fuel Research Tech. Pap.*, No. 23, 1929). Comparisons with the Fieldner method have shown that both methods give the same results. A new method which appears to yield more information regarding the nature of the fusion has been developed by Bunte and others (*Gas. u. Wasserf.*, 1928, **71**, 97; 1929, **72**, 838). In this method the movement of a cylinder of ash during fusion is recorded by a special mechanism. The shape of certain "fusion curves" is characteristic.

Further development in the measurement of fusion temperatures would appear to be imminent, but more attention should also be directed towards the correlation of the values to clinker formation in furnaces.

COAL CONSTITUENTS

Banded Constituents.—The tendency towards the evaluation of coals by chemical separation into constituents, as well as by proximate and ultimate analysis, has been steadily increasing as a result of the researches of Wheeler and Bone in this country, Fischer in Germany, and White and Thiessen in America. In the examination of coal seams use is also being made of the visible banded ingredients identified by Stopes (*Proc. Roy. Soc.*, 1919, *B*, **90**, 470), as the proportions of each which occur in a seam form a measure of the properties of the seam as a whole. A correlation by Thiessen and Francis with American conventions (*Bur. Mines Tech. Pap.*, 446, 1928) suggests classing vitrain with anthraxylon,

and clarain with attritus ; durain has no counterpart in American coals.

The identification of plant remains in coal has developed from an interesting research into a method which is capable of every-day application at a fairly low cost. The technique of making thin sections of coal is exemplified by Lomax (*Fuel Res. Tech. Pap.*, No. 11, 1925) in his examination of the Ravine seam.

Chemical Constituents. For the analysis of coal by means of solvents there are two main methods. The best known (Clark and Wheeler, *J. Chem. Soc.*, 1913, **103**, 1704) is that of extraction with pyridine, followed by a subsequent resolution of the extract by the solvent action of chloroform. By this means the coal is divided initially into three constituents, viz., insoluble, soluble in pyridine and insoluble in chloroform, and soluble in both solvents. The second method consists in the extraction of the coal with benzene under pressure and the separation of the extracts with petroleum spirit and alcohol (Bone and others, *Proc. Roy. Soc.*, 1922, *A*, **100**, 582 ; 1924, *A*, **105**, 608 ; Fischer and others, *Brennstoff-Chem.*, 1924, **5**, 299 ; 1925, **6**, 33).

Further details of the extensive researches which have been carried out in the last ten years cannot be included here, but the ultimate end would seem to be that the analysis of a coal will not be considered complete without information as to the proportions of its chemical constituents. The constituents to which are due the ability of the coal to form a hard coke are of special interest to the coke industry, and, without going to the extent of solvent extraction, the effect of these can be investigated by a method such as that of Charpy and Durand (*Compt. rend.*, 1920, **171**, 1358), as developed by Audibert (*Fuel*, 1926, **5**, 229). A cylinder of coal is heated in a tube in such a manner that fusion and intumescence can be observed by the movement of a balanced lever resting on the coal. The relation between fusion and decomposition temperatures has a bearing upon the treatment to which a coal must be subjected to form a hard coke.

" Rational " Analysis. The action of reagents upon coal has also disclosed a method of analysis whereby the coal is separated

into free hydrocarbons, resins, organised plant entities and ulmins (Cockram and Wheeler, *J. Chem. Soc.*, 1927, 700). A definite scheme of examination has been worked out by Francis and Wheeler (*J. Chem. Soc.*, 1928, 2967) and entitled "rational analysis." Hydrocarbons and resins are determined by extraction with pyridine, and the chloroform-soluble portion of the extract is taken to give the amount of these constituents. The ulmins are oxidised by atmospheric oxygen or by chemical oxidants, and their rate of oxidation is determined, as well as their solubility in alkali. Plant entities are determined by subtracting the amount of mineral matter from the residue left after extraction of the coal with a solution of nitric acid and potassium chloride made up according to the carbon content of the coal.

The hydrocarbons and resins are oil-yielding bodies, partly responsible for caking power. Their amount, like that of the organised plant remains, is not related to the degree of maturity of the coal, since the inclusion of such bodies in the coal at the time of its deposition is mainly adventitious. Ulmins, however, change progressively with the degree of "coalification" (Francis and Wheeler, *J. Chem. Soc.*, 1925, **127**, 2236), and therefore their nature has to be specified by a "reactivity index" based on the reactivity towards oxygen or on their solubility in alkali after regulated oxidation. As the ulmins constitute only a portion of the coal, the carbon content of the coal is only partially related to the reactivity index of the ulmins, and the determination of this index is therefore an essential key to the nature of the coals.

PULVERISED FUEL

In the examination of pulverised fuels perhaps the most important property to be determined is the size of particles. Screen analyses are difficult and not too satisfactory, but so far other methods suggested appear to be laborious. One of the earlier attempts to depart from the usual screen analysis is due to Sinnatt and Slater (*Fuel*, 1923, **2**, 142, 175), and takes the form in principle of a determination of the bulk density.

Alternative methods have so far been rather complicated, and

a simple but accurate method is urgently needed. Faber (*Z. Oberschles. Berg. u. Hütten. Ver.*, 1929, **68**, 404) has made use of the increase of density of a suspension over that of pure water and has observed photographically the decrease of density as the suspension settles. Calibration in terms of particles of known size allows of the measurement of the proportions of particles of different size. A simpler photographic method is due to Dunn (*Ind. Eng. Chem.* (*Anal.*), 1930, **2**, 59), who prepares enlarged photographs of dust samples and counts the grains of different sizes.

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CHAPTER XI

GAS ANALYSIS

By H. R. Ambler, B.Sc., F.I.C.

Measurement and Manipulation of Gas: Analysis of very Small Samples. *The Chemistry of Gas Analysis:* Carbon Dioxide and other Acidic Gases. Acidic Sulphur Gases. Oxygen. Ozone. Oxides of Nitrogen. Carbon Monoxide. Hydrogen and Methane. Hydrogen by Absorption. *Fractional Combustion Methods:* Carbon Monoxide in presence of Hydrogen and Paraffins. Hydrogen and Carbon Monoxide in presence of Methane. Nitrogen. Water Vapour. *Automatic Gas Analysis. Physical Methods:* Condensation Methods. Fractionation of Hydrocarbons. Condensation Method for very Small Quantities. Density Method. Velocity of Sound. Thermal Conductivity. Refractivity. Spectroscopy. Electrical Methods. *Summary of the Progress of Gas Analysis.* Bibliography.

THE quantitative analysis of gases was first put upon a working basis by Bunsen between the year 1839, in which his first paper on the subject was published (*Dinglers J.*, 1839, **71**, 321), and 1857, when his *Gasometrische Methoden* appeared. The general principle of Bunsen's method was to measure volumes of gas, and successively remove various constituent gases by selective chemical reactions, the amounts of these being measured by the changes in volume. These early experiments were characterised by extreme simplicity of apparatus and a corresponding complexity in manipulation: subsequent progress has consisted largely in the use of the same general principles under conditions giving greater ease and speed in working, attained largely by developments in apparatus. For this reason, gas-analysis has tended to stand apart from the general technique of analytical chemistry, and developments may be classified under two heads—improvements in the mechanical and physical methods of measuring volumes and manipulating samples, and the study of the chemical methods of dealing with the constituent gases.

Apart from this general technique, there are special methods for the estimation of individual gases, particularly tests for quantities which are too small for the ordinary volumetric method. In addition to these, there are the many physical and semi-physical methods that have been developed in recent years.

MEASUREMENT AND MANIPULATION OF GAS

In order to define any quantity of gas, its volume, pressure and temperature must be specified. In Bunsen's experiments all these three were variable, and were all measured. A step towards the modern measuring apparatus was made by Regnault and Reiset in 1849 (*Ann. Chim. Phys.*, [3], **26**, 333). In this apparatus, volume and temperature were kept constant and changes in pressure measured. The alternative principle, of measuring a varying volume at constant pressure, usually atmospheric, was adopted by Williamson and Russell (*J. Chem. Soc.*, 1864, **17**, 238) and by Doyère (*Ann. Chim. Phys.*, [3], **28**, 1). The "constant pressure" type of apparatus has the advantage over the "constant volume" type, of slightly simpler computation of results: instruments of secondary precision usually employ this principle, *e.g.*, Hempel's technical apparatus (Hempel, *Methods of Gas Analysis*, p. 21), and those of Orsat (*Chem. News*, 1874, **29**, 177) and Bunte (*J. für Gasbeleuchtung*, 1877, 447). The principle has also been embodied in a number of precision instruments, *e.g.*, those of White (*J. Amer. Chem. Soc.*, 1901, **22**, 343), Wilhelmy (*Z. angew. Chem.*, 1911, **24**, 874), and Perquin (*Chem. Weekbl.*, 1927, **24**, 321), and also the well-known Haldane apparatus, which remains popular as a precise and convenient instrument for a rather limited class of samples. (See Haldane, *Methods of Air-Analysis*, p. 14, and for later modifications, Carpenter, Fox and Screque, *Brit. Chem. Abstr.*, 1929, *A*, 1113, and McNair, *J. Soc. Chem. Ind.*, 1929, **48**, 374T.) For high-precision work, however, the tendency has been in favour of the "constant-volume" type, which is more sensitive in reading, and can generally be made simpler in construction. Apparatus of this type has been described by Hempel (*op. cit.*, p. 46). A very precise form has been designed by Huntly (see

Travers, *Study of Gases*, p. 69). A well-known form is the Bone-Wheeler apparatus (Bone, *J. Soc. Chem. Ind.*, 1906, **27**, 10). The constant-volume apparatus is also applicable to measurement on a gauge, which may be a convenience for rapid analyses of second-order accuracy (Ambler, *Analyst*, 1929, **54**, 521).

The third variant, temperature, is usually kept approximately constant by means of a water-jacket surrounding the measuring vessel. Any small changes that may then occur are second-order effects, and can, moreover, be precisely known and corrected for. Compensating devices to eliminate separate corrections for temperature changes have been described by Williamson and Russell (*J. Chem. Soc.*, 1864, **17**, 238), Pettersson (*Z. anal. Chem.*, 1886, **25**, 467), Haldane (*loc. cit.*) and Vilbrandt (*J. Ind. Eng. Chem.*, 1924, **16**, 936). These are designed for constant-pressure apparatus, and are usually incorporated in precision instruments of this type.

The only confining liquid suitable for precise work is mercury. Bone (*loc. cit.*) suggested as the best of bad alternatives a mixture of equal parts of glycerin and water. Other alternatives proposed are 22 per cent. sodium chloride solution (Hoffmann, *Z. angew. Chem.*, 1926, **39**, 23; *Chem. Abstr.*, 1926, **20**, 1370), and concentrated calcium chloride (Wolf and Krause, *Chem. Zentr.*, 1927, [II.], 1055). Tropsch (*Z. angew. Chem.*, 1926, **39**, 401; *Brit. Chem. Abstr.*, 1926, *B.*, 427) recommends the addition of a drop of sulphuric acid and also a drop of phenolphthalein in order to call attention in the event of the liquid becoming alkaline from contact with the reagents. For constant-volume work mercury is generally essential, as any other liquid would involve columns of enormous height.

In nearly all modern types of apparatus, measurement and absorption take place in different vessels. Absorption may take place in one special vessel into which the reagents are successively introduced. Donnelly, Foott and Reilly (*Sci. Proc. Roy. Dublin Soc.*, 1929, **19**, 165), with a modified Bone-Wheeler apparatus, have a simple method of filling the absorption pipette by means of a special six-way tap connecting it with bottles containing the reagents. Alternatively, a separate vessel may be kept filled with each reagent, and these connected in turn with the

measuring part of the apparatus (*e.g.*, Hempel, Sodeau). For rapid work of moderate precision, Wheeler (*Gas. J.*, 1922, **157,** 702) has a number of absorption-pipettes of the Hempel type connected permanently with the measuring system by means of a manifold with taps. A similar system is used in the Orsat apparatus, which has few advantages apart from its portability; a particular disadvantage is the difficulty of assisting absorption by agitating the reagents. To overcome this, many modifications of the absorption pipettes have been brought forward from time to time (see Dennis, *Gas-Analysis*, pp. 78–87; and, more recently, Aschoff, *Stahl. u. Eisen*, 1921, **41,** 1406; *Compt. rend.*, 1921, **173,** 237; *Chem. Ztg.*, 1921, **45**, 582; Neumann and Strahuber, *Arch. für das Eisenhüttenwesen*, 1929, **9,** 557 (dimensioned drawings given); and Bahr, *Chem. Fabr.*, 1929, p. 13). An absorption-pipette for more general purposes, designed to promote absorption by breaking the liquid up into spray, is described by Saunders (*J. Chem. Soc.*, 1923, **123,** 2826).

For very precise work, and also in dealing with small samples of gas, rubber connections are a source of appreciable error. In the latest forms of the larger types of instrument, these are usually replaced by flanged joints (see Grice and Payman, *Fuel*, 1924, **3,** 236; Donnelly, Foott and Reilly, *loc. cit.*); Dirken (*J. Sci. Instr.*, 1924, **2,** 55; *J. Soc. Chem. Ind.*, 1925, **44,** *B*. 192) has designed a novel apparatus with separate absorption pipettes which are successively connected with the measuring burette by faced joints. The instrument is made in two sizes, to take 5 and 15 c.c. respectively.

A small apparatus (Ambler, *Analyst*, 1929, **54,** 517), which I have found very satisfactory during some years' use, has the measuring and absorbing parts fused together as a one-piece unit. The elimination of rubber connections has made it possible to deal with smaller samples of gas than has been customary, without loss of accuracy. The measuring-chamber, in which gas is measured at constant-volume, consists of three bulbs, allowing for three alternative ranges of measurement; samples ranging from 0·25 c.c. to 15 c.c. can be dealt with, with an accuracy, in the case of the latter, of 0·1 per cent. An auxiliary manometer

connected with the absorption-chamber makes it possible to observe the progress of absorption without having to transfer the gas back to the measuring-chamber; this is useful with slow or untried absorbents. The apparatus is simple, strong, and easy to handle and read.* A much larger apparatus, of about 150 c.c. capacity, on somewhat similar lines, has been used at the Reichsanstalt (*Jahresber. der Chem.-Tech. Reichsanstalt*, 1926, **5**, 134).

An apparatus without either rubber connections or taps at which leaks could occur was devised by Doyère in 1850 (*loc. cit.*). This was very cumbersome; Hempel's apparatus "for exact analysis" was an advance on this, but was still very bulky, not easy to work, and required large quantities of mercury. A smaller and more convenient instrument without taps or rubber connections has been brought out by Chamberlin and Newitt (*Ind. Eng. Chem.*, 1925, **17**, 621; *Analyst*, 1925, **50**, 475). The volumes dealt with are 1 to 5 c.c.; the measuring system is of the Bone-Wheeler type.

Although the Dirken and Ambler instruments represent a tendency towards simplification and reduction in size, there has at the same time been a tendency in the opposite direction, due mainly to the increased use of fractional-combustion methods (*q.v.*). The large types of apparatus involve considerable physical work in manipulation, and, to cope with this, a number of devices have been brought out, mainly to raise and lower the mercury. Donnelly, Foott and Reilly (*loc. cit.*) do away with movable reservoirs, and force the mercury up directly by means of the water-supply. Blair and T. S. Wheeler (*J. Soc. Chem. Ind.*, 1928, **41**, 187T) make use of compressed air and a vacuum. A very ingenious electrically-controlled device is described by Perquin (*Chem. Weekblad*, 1927, **24**, 321). A simple arrangement for lifting mercury reservoirs by means of the water-supply is described by Tauch (*Ind. Eng. Chem.*, 1927, **19**, 1349).

Among portable instruments, the Orsat and the portable form of the Haldane apparatus remain in common use for different

* The instrument is obtainable from Messrs. A. Gallenkamp & Co. Ltd., Sun Street, E.C. 2.

classes of analysis. Other designs have been described by Murray (*J. Chem. Soc.*, 1925, **127**, 769) and Macdonald (*Gas. J.*, 1923, **164**, 779). The Ambler apparatus can also be made in a portable form. An entirely novel form of portable apparatus for the estimation of carbon dioxide in air is described by Lundegardh (*Arkiv. för Kemi. Mineralogi. och Geologi* (Stockholm), 1927, **9**, 1; *Chem. Zentr.*, 1928, [i.], 552); no confining liquid is used, the gas being introduced by means of a piston working on a rack. The gas is not transferred to an absorption vessel, but the absorbent (alkali solution on the surface of glass rods) lifted up into the space containing the gas sample. The instrument is apparently accurate to within 0·01 per cent. For further details, see the original paper (in German).

Analysis of very Small Samples.—Most of the instruments described above deal with samples ranging from 20 to 150 c.c. The Dirken apparatus can deal with 5 c.c., that of Chamberlin and Newitt with 1 c.c., and that of Ambler with about 0·25 c.c. For samples smaller than this, however, a different technique is necessary. It has generally been the practice to measure the gas at low pressure in a relatively large volume, with a McLeod gauge (Langmuir, *J. Amer. Chem. Soc.*, 1912, **34**, 1310; Ryder, *ibid.*, 1918, **60**, 1657; Prescott, *ibid.*, 1928, **50**, 3237). A much simpler procedure has been described by Reeve (*J. Chem. Soc.*, 1924, **125**, 1946) for samples of the order 0·05 c.c.: the gas is measured at atmospheric pressure in a water-jacketed capillary-tube. Apparatus on similar lines is described by Hamburger (*Z. anal. Chem.*, 1918, **57**, 121; *J. Soc. Chem. Ind.*, 1918, **37**, 446A) and by Christiansen (*J. Amer. Chem. Soc.*, 1925, **47**, 109; *Analyst*, 1925, **50**, 153).

THE CHEMISTRY OF GAS ANALYSIS

It may be observed that in the chemistry of gas analysis, perhaps even more than in other branches of analytical chemistry, the result of much of the investigation of recent years has been to reveal sources of error in methods that had generally been accepted as satisfactory, and, by eliminating such errors, to increase

the obtainable accuracy. This has been the case in nearly all the common processes of gas analysis.

Carbon Dioxide and Other Acidic Gases.—Since the introduction of liquid reagents for gas analysis the usual practice has been to absorb acidic gases with aqueous alkali. The most common solution is potassium hydroxide of 40 to 50 per cent. concentration. Bunte and Wunsch (*Gas-u. Wasserfach.*, 1923, **66**, 463; *J. Soc. Chem. Ind.*, 1923, **42**, 908A) point out, however, that the strong solution tends to absorb water vapour from the saturated gas and thus give high results; they recommend, where practicable, the use of a less concentrated solution (1 : 3). Solid alkali is used in the micro-analytical method of Reeve (*loc. cit.*) and in a specialised apparatus for the rapid routine estimation of carbon dioxide (Strache and Kling, *Feuerungstechnik*, 1922, **11**, 13; *J. Soc. Chem. Ind.*, 1922, **41**, 963A). It is also used when another gas is present which is soluble in aqueous solutions, such as nitrous oxide (*q.v.*) or acetylene (Friedrich, *Chem. Ztg.*, 1929, **53**, 706; *Brit. Chem. Abstr.*, 1929, *B*, 877).

The most important acidic gas met with in practice is carbon dioxide. Where more than one acidic gas is present, it is usual to absorb the whole in alkali, and to determine the other gases separately on separate samples. For precise measurement of very small quantities of carbon dioxide, see Johnstone and Walker (*J. Amer. Chem. Soc.*, 1925, **47**, 1807).

Hydrocyanic Acid. Seil (*Ind. Eng. Chem.*, 1926, **18**, 142; *Analyst*, 1926, **51**, 471) has worked out a simple and exact quantitative technique for this gas, based on the fact that it absorbs iodine in sodium bicarbonate solution (forming cyanogen iodide), and releases it on acidification. No other gas is known to behave similarly. Hydrogen sulphide, as well, absorbs iodine in sodium bicarbonate solution, but does not give it up on acidification. It is possible, therefore, to determine both these gases together by adding excess of iodine, and titrating first in bicarbonate and then in acid solution; this combined process, however, is not to be recommended, since it is particularly sensitive to experimental error. Hydrocyanic acid may be separated from cyanogen by absorption in slightly acidified silver nitrate. Among tests for

small quantities of these two gases, the Prussian blue test appears to be the only definitely conclusive one (Kolthoff, *Z. anal. Chem.*, 1918, **57**, 1; *J. Chem. Soc.*, 1918, *A*, [ii.], 138). In my experience, this test is made much more sensitive if the alkaline solution containing ferrocyanide is filtered free from excess iron before acidification; 0·01 milligram of HCN is easily detectable. The guaiacum test (see Cohn, *Indicators and Test Papers*, p. 192) is the most sensitive, but is liable to interference by ammonia, nitrous fumes, ozone and halogens. For other tests see Kolthoff (*loc. cit.*).

Acidic Sulphur Gases. Hydrogen sulphide and sulphur dioxide cannot exist together under ordinary conditions. Either may be determined with iodine, as in the classical method of Reich for sulphur dioxide (Dennis, p. 274). The latter gas may also be determined by absorption in alkali and titration of the sulphite: Haller (*J. Soc. Chem. Ind.*, 1919, **45**, 52T) finds that the addition of 5 per cent. of glycerin prevents oxidation of the sulphite to sulphate by the air. A third method, recently introduced, is based on the fact that bisulphites react with mercuric chloride quantitatively, according to the equation—

$$NaHSO_3 + HgCl_2 = HgCl(SO_3Na) + HCl$$

(Sander, *Chem. Ztg.*, 1921, **45**, 261, 554; *Z. anal. Chem.*, 1926, **68**, 50; Stuer and Grob, *Chem. Ztg.*, 1921, **45**, 553, 770). Dieckmann (*Papier-Fabrikant*, 1921, **19**, 285; *Analyst*, 1921, **46**, 517) describes a mixed-indicator method for the simultaneous determination of sulphite and sulphate. The addition of stannous chloride prevents oxidation of sulphite to sulphate (Dieckmann, *Chem. Ztg.*, 1921, **45**, 885). A physical method for sulphur dioxide is described later. For the micro-determination of traces of sulphur dioxide in air, see Rottinger (*Brit. Chem. Abstr.*, 1929, *A*, 1256), and for a general review of methods of determination of this gas, see *Z. anal. Chem.*, 1926, **68**, 49-54.

Carbon oxysulphide, COS, is absorbed easily by dilute alkali, but with difficulty by concentrated solutions. For analytical procedure based on this characteristic behaviour, see Stock and Kuss (*Ber.*, 1917, **50**, 159; *J. Chem. Soc.*, 1917, *A*, [ii.], 205).

Carbon disulphide can be determined by conversion into an

addition-compound with triethylamine, which can be weighed (Hegel, *Z. angew. Chem.*, 1926, **39**, 431). For very small quantities in air, Schvoinoff (*Ann. Chim. anal.*, 1929, **11**, 133; *Analyst*, 1929, **54**, 488) converts the carbon disulphide into potassium xanthate by means of alcoholic potash, and after neutralisation titrates with very dilute copper sulphate, with guaiacum as indicator. From 0·001 to 0·002 mgrm. is said to be detectable.

Oxygen. (1) *Absorption Methods.*—A wholly satisfactory absorbent for oxygen, that is to say, one which absorbs rapidly and completely, does not decompose on keeping, and evolves no gaseous products, is not yet known. Each of the absorbents used in practice has disadvantages of its own. The most generally useful reagent is alkaline pyrogallol. Hoffmann (*Z. angew. Chem.*, 1922, **33**, 325; *J. Soc. Chem. Ind.*, 1922, **41**, 613A) states that the maximum absorbing power is obtained when the ratio of pyrogallol to potassium hydroxide is 3 : 2. For practical use he recommends a composition of 20 per cent. of pyrogallol, 20 per cent. of potash and 60 per cent. of water. Sodium pyrogallate (Wey) and Goth, *Ber.*, 1881, **14**, 2649; Lewes, *J. Soc. Chem. Ind.*, 1891, **10**, 407; Berthelot, *Ann. Chim. Phys.*, 1898, **77**, 294; Shipley, *J. Amer. Chem. Soc.*, 1916, **38**, 1687; *J. Chem. Soc.*, 1916, *A*, [ii.], 671) appears to have a considerably higher absorbing power (Shipley, *loc. cit.*). Shipley recommended a very strong solution, made by mixing 10 c.c. of sodium hydroxide solution (1 : 1) with a solution of 10 grms. of pyrogallol in 4 c.c. of water. This solution is considered too viscous for practical use by Drakeley and Nicol (*J. Soc. Chem. Ind.*, 1925, **44**, 457T) and by Jones and Meighan (*J. Ind. Eng. Chem.*, 1919, **11**, 311). In my experience, however, it is quite convenient for use in small apparatus of the Ambler type. Hauser (*Bull. Soc. Chim.*, 1923, **33**, 1175) finds a solution of 10 grms. of pyrogallol in 50 c.c. of water, mixed with a solution of 95 grms. of sodium hydroxide in 150 c.c. water, to be rapid and satisfactory. Jones and Meighan (*loc. cit.*) recommend a solution of 250 c.c. of sodium hydroxide solution (1 : 1) mixed with 100 c.c. of pyrogallol solution (1 : 3). Sodium pyrogallate is said to be free from the drawback of slow absorption at low temperatures (*cf.* Hauser).

Ammonium pyrogallate is a satisfactory absorbent, but has no advantage over the sodium and potassium compounds to compensate for the inconvenience of evolution of ammonia (Drakeley and Nicol).

The chief disadvantage in the use of pyrogallol is its tendency to evolve carbon monoxide in the absorption process (Clowes, *J. Soc. Chem. Ind.*, 1891, **10**, 407; *Chem. News*, 1895, **72**, 288). There has been some divergence of evidence as to the magnitude of this effect. Shipley did not find that his strong solution gave off appreciable quantities. Anderson (*J. Ind. Eng. Chem.*, 1915, **7**, 587), using a solution made from 15 grms. of pyrogallol in 100 c.c. of potash of sp. gr. 1·55 (*i.e.*, about 1 : 1), found such error negligible in analyses of air. Trautz, Leonhardt and Scheuermann (*Z. anal. Chem.*, 1929, **78**, 341), in some precise analyses of gases containing about 0·2 per cent. of oxygen, do not seem to have found the effect appreciable. Jones and Meighan find that sodium pyrogallate made from alkali of less than 1·3 density does evolve carbon monoxide, and that all solutions give it off when the gas analysed contains more than 95 per cent. of oxygen. Drakeley and Nicol, who have made an elaborate investigation of the effect (*J. Soc. Chem. Ind.*, 1925, **44**, 457T; 1929, **48**, 62T; *Analyst*, 1925, **50**, 475; 1929, **54**, 306), find that for accurate analysis the effect is in no case negligible. My own experiments lead me to the same opinion. The effect is increased by a high oxygen content; samples containing more than 20 per cent. should first be diluted with nitrogen, even in industrial analyses (Drakeley and Nicol). The effect is minimised by agitation of the solution during absorption; this is stated to be a very important factor, and for this reason, it is considered that pyrogallol is always unsuitable for use in the Orsat apparatus or other types that cannot be agitated. The effect is also minimised by high concentration of pyrogallol and by high ratio of alkali to pyrogallol. Shipley's solution is stated to evolve considerable quantities of carbon monoxide. The objection raised on the same ground by Hempel (*Methods of Gas Analysis*, p. 115) to the use of potassium hydroxide purified by alcohol does not appear valid, according to Anderson.

An alternative absorbent, free from this source of error, is sodium hydrosulphite, $Na_2S_2O_4$ (Franzen, *Ber.*, 1896, **39**, 2069). The rate of absorption is not greatly affected by temperature (Haaser, p. 1176). Absorption is, however, considerably slower than with pyrogallol, in spite of statements that "absorption is in all cases complete in five minutes" (Treadwell, *Analytical Chemistry* (1924), Vol. II., p. 654). The absorption may, however, be greatly accelerated by the addition of sodium anthraquinone β-sulphonate (Fieser, *J. Amer. Chem. Soc.*, 1924, **46**, 2639; *Analyst*, 1925, **50**, 89). The solution recommended for general purposes consists of 16 grms. of sodium hydrosulphite, 6·6 grms. of sodium hydroxide and 2 grms. of the sulphonate with 100 c.c. of water. Vigorous shaking is unnecessary; absorption is still slower than with pyrogallol when used in the ordinary way in absorption pipettes, but for bubblers is stated to be more rapid. For this use a composition of 16 grms. of sodium hydrosulphite, 13·3 grms. of sodium hydroxide and 4 grms. of the sulphonate, with 100 c.c. water, is recommended. When the solution is exhausted, the colour changes from blood-red to brown. The chief drawback of this, and of all hydrosulphite solutions, is that they spontaneously decompose, particularly in hot weather; according to Kruse (*J. Pharmacol. Proc.*, 1925, **25**, 151; *Analyst*, 1926, **51**, 214), who appears to have discovered independently the reagent described above, it becomes very sluggish on keeping for a fortnight; this is also my own experience.

Phosphorus has been employed from early times as an absorbent for oxygen. Disadvantages are, that the action is inhibited if the oxygen content exceeds 75 per cent., and also, partly or wholly, by olefines, acetylenes, alcohol and ammonia. According to Hempel (*Methods of Gas Analysis*, p. 124), 0·25 per cent. of ethylene is enough to inhibit absorption.* This is denied, however, by Ott (*Helv. Chim. Acta*, 1924, **7**, 886; *J. Chem. Soc.*, 1924, *A*, [ii.], 875). Higher paraffins in large quantities have the same effect (Burrell and Seibert, *U.S. Bur. Mines Bull.* 197, p. 92). According

* The figure of 0·04 per cent. ascribed to Hempel (Treadwell, 1924, Vol. II., p. 654), would appear to arise from a misreading of "1 400" (*cf. Methods of Gas Analysis*, p. 124; *Gasanalytische Methoden*, p. 138).

to Ott, benzene up to 6 per cent. does not inhibit the absorption. Absorption is also stated to be incomplete in the residual gases after exploding for hydrogen, etc., in gas analysis, and in the gases from engine exhausts (Holmes, *J. Ind. Eng. Chem.*, 1923, **15**, 357; *J. Soc. Chem. Ind.*, 1923, **43**, 475A), this being presumably due to the formation of traces of inhibiting gases. The gas can, however, be "activated" by first passing a small quantity of it into bromine water (*loc. cit.*)

The phosphorus method is sometimes found convenient for the Orsat apparatus, for which pyrogallol and hydrosulphite have particular disadvantages. In the design of a pipette for phosphorus, it is important for the phosphorus to be near the bottom of the gas-space (Neumann and Strahuber, *Arch. für das Eisenhüttenwesen*, 1929, **9**, 557; diagrams of suitable pipette given). Phosphorus dissolved in castor oil may be used in place of the solid (Centnerszwer, *Chem. Ztg.*, 1911, **34**, 494; Dennis, *Gas-Analysis*, p. 166). Phosphorus in this form can apparently deal with gases of much higher oxygen-content than the solid element.

Determination by Combustion. A precise and convenient method for the determination of small quantities of oxygen in hydrogen, or in inert gases containing hydrogen, is by combustion with platinum wire at dull-red heat in a slow-combustion pipette (Ambler, *Analyst*, 1930, **55**). One minute's heating of the wire is enough. An accuracy of 0·01 per cent. is attainable. Provided the wire does not get above dull-red heat, methane does not interfere. In the presence of carbon monoxide the precision is less, owing to the oxidation of some of the latter, but with the wire at a temperature at which it is just visibly red, the proportion of carbon monoxide oxidised is small, provided the amount of oxygen to be measured does not exceed 1 per cent. Under these conditions, an accuracy of 0·05 per cent. is attainable. Carbon dioxide does not interfere. Geissler (*Z. angew. Chem.*, 1925, **38**, 948) has described a special apparatus working on the same principle for estimating either oxygen in hydrogen or carbon monoxide in air. Steuer (*Chem. Ztg.*, 1925, **49**, 713; *Analyst*, 1925, **50**, 525), for less precise work, employs a quartz

capillary filled with platinum wire. Trautz and Kipphan (*Z. anal. Chem.*, 1929, **78**, 350) absorb oxygen by means of iron wire electrically heated to redness.

Tests for Small Quantities of Oxygen.—Schulek (*Z. anal. Chem.*, 1926, **68**, 22) describes a method for the qualitative detection of oxygen, based on the manganous hydroxide method commonly used in water analysis (Winkler, *Ber.*, 1888, **21**, 2843; Treadwell, Vol. II., p. 654): 0·02 c.c. in 100 c.c. is detectable. The application of the principle to quantitative estimation is, however, less satisfactory, owing to the difficulty of getting the oxygen into solution where it can react. Burrell and Seibert (*loc. cit.*, p. 94) find that with a sample of 100 c.c. the gas should be shaken for fifteen minutes with 15 c.c. of alkali and 25 c.c. of manganous chloride solution. Where large quantities of gas are available it may be bubbled through the reagent at a rate not greater than 5 c.c. per minute: at least three bubblers in series should be used. Even then results tended to be low (*loc. cit.*). Mr. Sutton and I found that when 3,000 c.c. of nitrogen containing 5 c.c. of oxygen were subjected to violent mechanical shaking for three hours with 100 c.c. of the reagent, only 80 per cent. of the oxygen was taken up.

Very small traces of oxygen can be detected by the glowing of phosphorus in the dark. Heyne (*Z. angew. Chem.*, 1925, **38**, 1099; *Z. anal. Chem.*, 1928, **73**, 153) states that 5×10^{-5} per cent. of oxygen can be detected in inert gases by this means. Carbon monoxide, ethylene and other organic gases interfere.

In exceptional cases, where other methods cannot be used, oxygen may be determined by the addition of a known volume of nitric oxide and observing the contraction. There is usually enough moisture in the burette to absorb the nitrogen peroxide produced. The principle is applied to minute quantities of oxygen by Schmalfuss and Werner (*J. prakt. Chem.*, 1925, **111**, 62; *J. Soc. Chem. Ind.*, 1925, **44**, *B*. 989), who describe a simple qualitative technique, using the diphenylamine colour test for nitrogen peroxide.

The same authors (*Ber.*, 1925, **58**, 71) make use of the darkening of alkaline pyrogallol for a colorimetric test which will detect

0·008 per cent. of oxygen. I have used ammoniacal cuprous chloride in a similar way; the gas was slowly bubbled through the cuprous chloride in a modified Duboscq colorimeter; 0·01 c.c. of oxygen was detectable, and further oxygen increased the depth of colour in a linear relation.

A very sensitive colour reagent for oxygen is obtained by adding alkali to a solution of ferrous sulphate and catechol (Binder and Weinland, *Ber.*, 1913, **46,** 255). Oxygen gives an intense red colour. According to Heyne (*Z. angew. Chem.*, 1925, **38,** 1099; *Z. anal. Chem.*, 1928, **73,** 153), $1{\cdot}4 \times 10^{-3}$ per cent. of oxygen was detected by this test (in 17 litres of argon). Water and carbon dioxide do not interfere. For other colour tests see Hofer and von Wartenberg (*Z. angew. Chem.*, 1925, **38,** 9; *J. Soc. Chem. Ind.*, 1925, **44,** *B*, 88) and Efimov (*Biochem. Z.*, 1925, 155, 371; *Analyst*, 1926, **51,** 213).

Tungsten wire at very dull red-heat is attacked by oxygen, producing surface colorations; 2×10^{-4} per cent. can be detected. Water and carbon dioxide must be first removed (Heyne, *loc. cit.*).

Ozone.—A sensitive method for the estimation of traces of ozone in air, based on the bleaching of fluorescein, was brought out by Benoist in 1919 (*Compt. rend.*, 1919, **168,** 612; *Analyst*, 1919, **44,** 183). Egorow (*Z. Unters. Lebensm.*, 1928, **56,** 355; *Analyst*, 1929, **54,** 189) reverses the technique by substituting for fluorescein the non-fluorescent leuco-compound fluorescin, which is converted by ozone to fluorescein. Hydrogen peroxide and oxides of nitrogen do not interfere. The fact that fluorescein itself is attacked by ozone, but more slowly than fluorescin, appears to limit the accuracy of Egorow's method (see Allen, *Ind. Eng. Chem. J.*, (*Anal. Ed.*), 1930, **2,** 55). As regards Benoist's method, crystal violet and methyl violet are almost as sensitive as fluorescein (Allen, *loc. cit.*). For other tests, see von Wartenberg and Podjaski (*Z. anorg. Chem.*, 1925, **148,** 391; *J. Soc. Chem. Ind.*, 1925, **44,** *B*, 989), McDonnell (*Ind. Eng. Chem.*, 1926, **18,** 135; *J. Soc. Chem. Ind.*, 1926, **45,** *B*, 273) and Allen.

Oxides of Nitrogen.—*Nitrous Oxide.*—The estimation of this gas has always presented difficulty, because, while no known

reagent will absorb it completely, all aqueous solutions dissolve it slightly. No definite chemical test has yet been discovered. The use of alcohol as a solvent (Lunge, *Ber.*, 1881, **14**, 2188) is not, in my experience, very satisfactory, repeated shaking with successive quantities of alcohol being necessary for complete absorption, whilst the solubility of other gases in alcohol is not negligible. Where there are a number of other gases present, it is best to remove nitrous oxide by condensation with liquid air; carbon dioxide, if present, may be previously removed by solid alkali. For small quantities of nitrous oxide, the condensation method is the only satisfactory one. The chemical methods described below may then be applied to the gas concentrated and purified by condensation. An exhaustive study of these chemical methods has been made by Menzel and Kretschmar (*Z. angew. Chem.*, 1929, **42**, 148; *Brit. Chem. Abstr.*, 1929, *A*, 414). The conclusions arrived at are: (i.) Winkler's method of exploding with oxygen is satisfactory if the nitrous oxide content is not more than 10 per cent.; above this concentration, higher oxides are produced. (ii.) Thermal decomposition by means of electrolytic gas is satisfactory for gases containing up to 5 per cent. (iii.) The most reliable procedure is Bunsen's method of reduction with hydrogen, either by explosion, or by slow-combustion, as in a platinum-filled quartz capillary. This is satisfactory for all proportions of nitrous oxide. For the estimation of oxygen in the presence of nitrous oxide, the same investigators find phosphorus to be satisfactory. Physical methods of analysis (*q.v.*) are of particular applicability to nitrous oxide.

Nitric Oxide.—The usual absorbent for this gas is ferrous sulphate. Divers (*J. Chem. Soc.*, 1899, **75**, 82) suggested an alkaline solution of sodium sulphite. Moser, however (*Z. anal. Chem.*, 1914, **50**, 401; Dennis, p. 220), finds this to be a very slow absorbent, and not superior to ferrous sulphate; this is my own experience also. Barnes (*J. Soc. Chem. Ind.*, 1926, **45**, 259T) finds that pumice soaked in a 40 per cent. solution of sodium sulphite, made strongly alkaline, to be an efficient absorbent.

Nitrogen Peroxide.—Nitrogen peroxide cannot be confined over mercury, as it attacks it. Whittaker, Lundstrom and Merz

(*Ind. Eng. Chem.* (*Anal. Ed.*), 1930, **2**, 15) estimate it in mixtures with air by measuring the volume of the mixture confined over a mineral oil, "Nujol," at 156°, at which temperature the oil is mobile and the N_2O_4 practically completely dissociated to NO_2. The nitrogen peroxide is then absorbed in concentrated sulphuric acid, and the residual air measured over mercury at atmospheric temperature. For the detection and estimation of very small quantities, the Griess-Ilosvay test, or the substitute consisting of *p*-nitraniline and α-naphthol (Moir, *Analyst*, 1921, **46**, 517) provides about as sensitive a test as is known for any gas. For the exact estimation of small quantities, the nitrous acid which is produced together with nitric acid when nitrogen peroxide reacts with water, is oxidised, and the total nitric acid determined by the phenolsulphonic acid method (Allison, Parker and Jones, *U.S. Bur. Mines, Tech. Paper* 249). Francis and Parsons (*Analyst*, 1925, **50**, 262) find that nitrogen peroxide, etc., are not completely removed by scrubbing with hydrogen peroxide and alkali, as recommended by the above authors, but may be completely taken up by shaking in a bottle with acid hydrogen peroxide for about three hours. For the estimation of very small quantities (less than one part in a million) in air, the nitrous fumes are concentrated by condensation in liquid air and subsequent evaporation into an evacuated bottle.

Chlorine. Porter (*Ind. Eng. Chem.*, 1926, **18**, 730; *Analyst*, 1926, **51**, 476) describes a very sensitive method for detecting traces of chlorine in air, based on the colour reaction with *o*-tolidine. A sensitiveness of 0·01 part in a million is claimed.

Carbon Monoxide, Hydrogen, and Methane

Carbon Monoxide. *Absorption Methods.* The estimation of the above three gases is conveniently treated as a whole, since the estimation of one usually affects that of the others. The classical method, and that still most commonly used, is to absorb carbon monoxide with cuprous chloride, and to explode the hydrogen and methane with oxygen or air. The main source of error in this procedure is incomplete absorption of carbon monoxide;

this makes the methane figure too high, and the hydrogen correspondingly low. Cuprous chloride, which is insoluble in water, is generally used either in ammoniacal or in hydrochloric acid solution. There are other solutions in which it is soluble, such as saturated marine salt (le Chatelier, *Leçons sur le Carbone*, 1908, p. 50) or concentrated calcium chloride solution (Treadwell, Vol. II., p. 657), but these would appear to be of little practical use. The acid solution is easier than the ammoniacal to prepare, and is stated to be more rapid (Hauser, *Bull. Soc. Chim.*, 1923, **33**, 1185). It appears, however, to absorb completely in no circumstances, even unused solutions leaving considerable proportions of carbon monoxide unabsorbed. Solutions that have absorbed carbon monoxide are less satisfactory still (Ambler, *Analyst*, 1925, **50**, 167). The ammoniacal solution is a more complete absorbent; a properly prepared solution that has not already absorbed more than 5 per cent. of its volume of carbon monoxide is adequate for most purposes (*loc. cit.*). For accurate analysis, it is advisable to use three successive lots of the reagent in order to ensure that the final stage of the absorption is done by a fresh solution. Prolonged shaking beyond one minute is not generally effective in causing further absorption (*loc. cit.*). Solubility of other gases, usually nitrogen, in cuprous chloride solutions is not to be overlooked (Ott, *Helv. Chim. Acta*, 1924, **7**, 886; *Z. anal. Chem.*, 1926, **68**, 239). The amount of reagent should be kept small. I find it advisable to saturate with nitrogen before use. Moser and Henika (*Z. anal. Chem.*, 1926, **67**, 148; *Analyst*, 1926, **51**, 266), also find the ammoniacal solution superior to the acid, and recommend a composition of 11 to 12 parts of cuprous chloride, 13 to 14 of ammonia (as NH_3) and 74 to 76 of water. Small quantities (3 per cent.) of stannous chloride improve the absorbent, but large quantities are detrimental (*loc. cit.*).

A reagent which absorbs carbon monoxide completely was brought out by Damiens in 1924 (*Compt. rend.*, 1924, **178**, 849, 2176; *Analyst*, 1924, **49**, 298), consisting of a suspension of cuprous sulphate in sulphuric acid. This gave complete absorption in the Orsat apparatus (La Condamine, *loc. cit.*). The reagent was

improved by Lebeau and Bedel (*Compt. rend.*, 1924, **179**, 108; *Analyst*, 1924, **49**, 451) by the addition of β-naphthol, whereby the cuprous sulphate is brought into solution. Absorption by this reagent is complete, even when it has already absorbed five times its volume of carbon monoxide (Ambler, *loc. cit.*). The gas should be washed with alkali after treatment with the reagent, as small quantities of acidic gases are usually produced. A disadvantage is the slowness of absorption, which may take up to thirty minutes; by using the reagent at 60°, however, this time is reduced to three minutes (Sutton and Ambler, *Analyst*, 1925, **50**, 172). Hydrogen and saturated hydrocarbons are not affected; the reagent absorbs twenty times its volume of ethylene. Mercury is not attacked (Lebeau and Bedel). It is said to work much better if the cuprous oxide used in the preparation is prepared by the wet method (La Condamine, *Glückauf*, 1925, 346; *Chem. Ztg.*, 1925, **49**, 405). See also Schläpfer and Hofmann (*Monats. Bull. Schweiz. Ver. Gas-Wasserfachmannern*, 1927, **7**, 293, 349; *Chem. Zentr.*, 1929, [i.], 3013; *Brit. Chem. Abstr.*, 1930, *B*, 5).

Other absorbents that have been proposed for various purposes are cuprous ammonium formate and carbonate (Larson and Teitsworth, *J. Amer. Chem. Soc.*, 1922, **44**, 2878; *Analyst*, 1923, **48**, 138). According to Piutti (*Giorn. Chim. Ind. Appl.*, 1923, **5**, 70; *Analyst*, 1923, **48**, 230), a mixture of cuprous chloride, fir charcoal, pumice and soda-lime is an efficient absorbent for the purpose of protection against poisoning, and is also stable in air.

Schläpfer and Hofmann (*loc. cit.*) recommend a suspension of iodine pentoxide in 10 per cent. oleum, at ordinary temperatures for the determination of carbon monoxide in quantities above 0·2 per cent. Hydrogen, oxygen and methane are not affected. I have tried a suspension of iodine pentoxide in concentrated sulphuric acid, but find it slow and incomplete in its action. Part of the carbon dioxide produced dissolves in the acid and the remainder has to be absorbed by alkali.

Special Methods. The property of carbon monoxide of reducing ammoniacal silver solutions is applied by Manchot and Scherer (*Ber.*, 1927, **60**, 326; *Z. anal. Chem.*, 1928, **75**, 40; *Brit. Chem. Abstr.*, 1927, *A*, 331) to a titrimetric method of estimation. The

gas sample is shaken in a stoppered flask with a solution made from 50 c.c. of 0·1*N*-silver nitrate, 50 c.c. of 0·15*N*-sodium hydroxide and enough pyridine (which is found preferable to ammonia) to dissolve the silver hydroxide precipitate. The solution is then diluted, acidified with acetic acid, and the excess of silver titrated. Hydrogen, methane and ethylene do not interfere. The fact that ethylene does not interfere may make this method of value for carbon monoxide estimation in gases containing ethylene, since ethylene is absorbed by all the CO absorbents described previously. The same authors (*op. cit.*) have worked out an alternative method based on the reduction of gold chloride at 60° to 70° to gold. Methane does not interfere; hydrogen, however, restricts the action, and ethylene reduces the gold chloride.

An ingenious method for the estimation of carbon monoxide in hydrogen is described by Larson and Whittaker (*Ind. Eng. Chem.*, 1925, **17**, 317; *J. Soc. Chem. Ind.*, 1925, **44**, *B*, 400). The gas is passed over a nickel oxide catalyst at 300°, which converts it into methane and water, according to the equation —

$$CO + 3H_2 = CH_4 + H_2O.$$

Water, which is a measure of the carbon monoxide, is determined by the dew-point method. For this reason, the technique is suited only to cases in which the partial pressure of carbon monoxide is less than the saturated vapour pressure of water. Results appear to be accurate to about 0·03 per cent. of the total gas. The same reaction is employed by Schuftan (*Z. angew. Chem.*, 1926, **39**, 276; *Z. anal. Chem.*, 1928, **75**, 42) who claims that the method is more accurate than absorption methods. If the temperature of the reaction chamber exceeds 300°, or if the carbon monoxide content exceeds 10 per cent., error results owing to formation of carbon dioxide.

Tests for Small Quantities of Carbon Monoxide. Methods with iodine pentoxide and with copper oxide are described under "Fractional Combustion Methods"; for laboratory work, these are probably the most satisfactory. For tests in mines, etc., however, a number of rapid and handy tests have been worked out. The use of ammoniacal silver solution, which shows a dark colour

with small quantities of carbon monoxide, was first suggested by Berthelot (*Compt. rend.*, 1891, **112**, 597). The addition of alkali accelerates the action (Thiele, see *Jahresber. der. Chem.-Tech. Reichsanstalt*, 1924, **4**, 98; Kast and Selle, *Glückauf*, 1926, **62**, 804; *Gas-u. Wasserfach.*, 1926, **69**, 812). The procedure developed by the Reichsanstalt (*loc. cit.*) is to take 1 c.c. of the solution in a sealed, evacuated glass tube. To test for carbon monoxide in air, the tube is broken, and the time taken for the dark tinge to appear noted: 1·6 per cent. shows in seven seconds and 0·05 per cent. in eighty seconds. Hydrogen and methane have no effect, ethylene in quantities up to 2 per cent., and proportionately small traces of acetylene do not interfere (*Jahresber.*, 1927, **6**, 103). This method is preferred to the palladium chloride test, which is also described (*Jahresber.*, **4**, *loc. cit.*). See also Nanckoff (*Z. für Schiess-u. Sprengst.*, 1909, **4**, 242; Nowicki, *Chem. Ztg.*, 1911, **35**, 1120; Desgrez, Labat and Savès, *Chim. et Ind.*, 1921, **5**, 473; *Chem. Zentr.*, 1921, [iv.], 317; Wein, *Glückauf*, 1925, **61**, 1623; *Stahl u. Eisen*, 1926, **56**, 1436). According to Wein, 0·02 to 0·03 per cent. of carbon monoxide is detectable by this test. See also Schläpfer and Hofmann (*loc. cit.*).

Iodine pentoxide and concentrated sulphuric acid soaked into pumice gives colours with carbon monoxide, varying according to the amount of the latter present; 0·005 per cent. in a sample of 500 c.c. is said to be detectable (Hoover, *J. Ind. Eng. Chem.*, 1921, **13**, 770; *Analyst*, 1921, **46**, 470). The reaction is exothermic, and will take place at temperatures as low as −10°. Unsaturated hydrocarbons, gasoline vapour, hydrogen sulphide, arsine, and hydrogen cyanide react similarly and may be tested for with the reagent; in testing for carbon monoxide, these may be previously absorbed by active charcoal. Hydrogen, methane, sulphur dioxide, nitric acid and ammonia have no action (Hoover). When used under parallel conditions (*i.e.*, in small evacuated tubes) this test appears to be less sensitive than the two mentioned above (*Jahresber.*, **4**, *loc. cit.*). See also Lamb, Bray and Frazer (*J. Ind. Eng. Chem.*, 1920, **12**, 213); Kast and Selle (*Gas-u. Wasserfach.*, 1926, **69**, 819). "Hoolamite" is a commercial name for this reagent.

The most positive test of all is the old and tried blood-test, based on the observation of specific bands in the absorption spectrum of blood which has absorbed carbon monoxide (see Hempel, p. 165; Dennis, p. 226). Refinements introduced by Nicloux (*Compt. rend.*, 1925, **180**, 1750) make the test sensitive to 1 part in 100,000 on a sample of 500 c.c.c. See also Stavorinus (*Het Gas*, 1927, **47**, 162; *Chem. Zentr.*, 1927, [i.], 2259), who considers this the only satisfactory test. A new test, also with blood, has been developed by Sayers, Yant and Jones (*U.S. Bur. Mines Tech. Paper* 375 [1925]; *Bull.* 197, p. 66). This is based on the fact that when blood containing carbon monoxide is diluted with water and treated with a solution of gallotannin and pyrogallol a red suspension is formed, whilst with normal blood, the suspension is brownish-grey.

The Siemens carbon monoxide indicator (*q.v.*) is very convenient to use for quantities above 0·05 per cent. (*Jahresber.*, **4**, *loc. cit.*).

Hydrogen and Methane. *Determination by Complete Combustion.*—After removal of carbon monoxide, it is usual to burn hydrogen and methane with excess of oxygen. If methane is known to be absent, absorption of carbon monoxide may be omitted, and this gas burned together with the hydrogen, subject to the restrictions described below. If the amount of oxygen used in the combustion is measured, it is possible to estimate all three gases together by complete combustion, but for precise work this procedure contains too many sources of error for it to be recommended.

Combustion by explosion is simple and, within certain limits of composition, very satisfactory. If the proportion of combustible gas is too great, nitrogen (which is usually present) becomes oxidised, and the contraction is too great; in addition to which, nitrogen peroxide produced may be registered as methane. The limits within which accurate results can be obtained seem to vary considerably according to the type of explosion vessel used. In the case of hydrogen, according to my own experiments with spherical bulbs, oxidation of nitrogen is negligible if the proportion of hydrogen in the explosive-mixture does not exceed 20 per cent. (*Analyst*, 1930, **55**, 55). If the proportion of

hydrogen is too small, combustion is incomplete; stress must be laid upon the fact that a definite visible explosion is no criterion of complete combustion; the lower limit under the same conditions is about 10 per cent. (*loc. cit.*). Jones and Parker (*J. Ind. Eng. Chem.*, 1921, **13,** 1164), with a Morehead burette, put the upper limit considerably higher; White (*J. Amer. Chem. Soc.*, 1901, **23,** 476), with Hempel pipette, finds an upper limit of 16·5 per cent. When the explosion takes place in a eudiometer-tube, that is, under approximately "constant-pressure" conditions, the lower and upper limits of 14 per cent. and 26 per cent., determined by Bunsen, would appear to stand (see Hempel, p. 101).

With methane, in the absence of other combustible gas, the upper limit is 8·3 per cent. for "constant-volume" conditions in spherical bulbs (see Hauser, *Bull. Soc. Chim.*, 1923, **33,** 1207). Under "constant-pressure" conditions, Bunsen's upper limit of 9·1 per cent. may be taken (Hauser). The appearance of the explosion-flame is a guide to whether oxidation is likely to have taken place; a reddish flame is a sign that the explosion was satisfactory, a blue flame indicates incomplete combustion, and a yellow flame probable oxidation of nitrogen (Hauser).

It appears that hydrogen cannot be accurately determined by explosion, in the presence of homologues of methane, since small quantities of hydrogen escape combustion (Misteli, *J. für Gasbeleuchtung*, 1905, **48,** 802; *Chem. Zentr.*, 1905, [ii.], 1075; *J. Chem. Soc.*, 1905, *A*, [i.], 849). As regards methane itself, my own experiments (*loc. cit.*) indicate that whereas, whether methane be present or not, minute traces of hydrogen (up to 0·05 per cent. of the total gas) always escape combustion, the presence of methane does not augment this effect.

As regards apparatus for explosion experiments, the usual way of igniting the mixture is by a spark across stout platinum electrodes fused into a glass bulb. Hauser (*loc. cit.*, p. 1204) ignites by means of a heated platinum spiral; if the gas does not explode, the combustion can be forthwith carried out by maintaining the spiral at red or yellow heat, as described below. It is desirable for the platinum to be easily replaceable; the usual form of accessible spiral, such as that in the Haldane apparatus, is, on the

other hand, not suitable for any but the mildest explosions. The combustion-pipette described by Weaver and Ledig (*J. Ind. Eng. Chem.*, 1920, **12**, 368), in which a replaceable spiral is fitted through side-limbs might possibly be suitable for this double purpose (see also Hauser, *loc. cit.*). An iron explosion-vessel described by Tauch (*Ind. Eng. Chem.*, 1927, **19**, 1349) may possibly be useful in technical work, although for any explosions which may occur in precise analysis, glass is amply strong.

The slow-combustion method by means of heated platinum wire is suitable for all ranges of composition (see Dennis and Hopkins, *J. Amer. Chem. Soc.*, 1899, **21**, 398; Dennis, *op. cit.*, pp. 147 *et seq.*). As regards possible errors due to oxidation of nitrogen, it appears from the experiments of Rhodes (Dennis, p. 153), Jones and Parker (*loc. cit.*), and myself (*loc. cit.*) that, in spite of the large errors recorded by White (*loc. cit.*), such action is negligible with the wire at bright yellow heat for three minutes. At white heat, however, the effect may be sufficient to cause appreciable error.

The size of wire which I have found convenient is 0·125 mm. diameter (also by Weaver and Ledig, *loc. cit.*). Dennis and Hopkins recommended 0·25 mm. For a modified Orsat apparatus, Bahr (*Chem. Fabr.*, 1929, 13) has suggested wire of 0·5 mm., taking a current of 12 to 15 amperes. Slow combustion may also be carried out in the platinum-filled capillary (Levy, *J. Soc. Chem. Ind.*, 1912, **31**, 1153; Hempel, *Z. angew. Chem.*, 1912, **25**, 1841).

The method occasionally recommended in the past, of effecting the combustion of hydrogen and methane in non-explosive mixtures by means of continued sparking is, in my experience, wholly inadmissible except for the roughest of analyses, owing to the very considerable oxidation of nitrogen that takes place.

Hydrogen by Absorption. Hydrogen may be estimated by absorption in colloidal platinum (Paal and Hartmann, *Ber.*, 1910, **43**, 243; Brunck, *Chem. Ztg.*, 1910, **34**, 1313). Absorption takes 10 to 20 minutes (Dennis, p. 153). In view of the simplicity, speed, and accuracy of the combustion methods, the applicability of this method would seem to be confined to special cases, such

as the determination of hydrogen in the presence of paraffins in apparatus that is not fitted for fractional-combustion methods.

Bosshard and Fischli (*Z. angew. Chem.*, 1915, **28**, 365) proposed as an absorbent for hydrogen, sodium oleate containing 3 per cent. of nickel; from the experiments of Anderson and Katz (*J. Ind. Eng. Chem.*, 1918, **10**, 23), however, it would not appear to be very satisfactory.

Tests for Small Quantities of Hydrogen. Heyne (*Z. angew. Chem.*, 1925, **38**, 1099) finds that 0·015 per cent. of hydrogen in 100 litres of an inert gas passed over wire copper oxide gives a reddening detectable to the naked eye. Smaller quantities than this gave reddening effects detectable by more careful scrutiny. A more sensitive test is to convert the hydrogen into hydrogen chloride by means of chlorine, and after removal of the excess chlorine with silver gauze, to estimate the chloride: 0·005 per cent. was easily detectable by this means (*loc. cit.*). The same author (*Z. anal. Chem.*, 1927, **70**, 179; *Brit. Chem. Abstr.*, 1927, *B*, 217) has more recently described a modification of this, whereby the hydrogen is converted into hydrogen chloride by means of nickel chloride at 600°; 0·001 per cent. was detectable. Shikata (*J. Pharmacol. Soc. Japan*, 1927, **49**; *Chem. Zentr.*, 1927, [ii.], 295) describes colour tests for reducing gases, based on the catalytic reduction of molybdenum trioxide. 0·01 per cent. of hydrogen was detectable.

FRACTIONAL COMBUSTION METHODS

Carbon Monoxide in the Presence of Hydrogen and Paraffins.— For the precise estimation of hydrogen, carbon monoxide and methane in the presence of each other, increasing use is being made of fractional combustion methods, that is to say, processes in which one gas is oxidised, while another is unaffected. Carbon monoxide can be completely oxidised in the presence of hydrogen and methane by means of iodine pentoxide; this was first made use of by de la Harfe and Reverdine in 1888 (*Chem. Ztg.*, 1888, **12**, 1726) and developed by Nicloux (*Compt. rend.*, 1898, **126**, 746) and by Gautier (*ibid.*, 931). Since then, a very large amount

of research has been done on the technique of the process, to increase the precision, and to determine the extent to which other gases interfere. The reaction proceeds in accordance with the equation—

$$5CO + I_2O_5 = 5CO_2 + I_2.$$

The carbon monoxide is determined by collection of the iodine liberated, or of the carbon dioxide. Levy and Pecoul (*Compt. rend.*, 1906, **142,** 162) collected the iodine in chloroform and observed the depth of colour of the solution produced; more usually the iodine is collected in potassium iodide solution, which is subsequently titrated with thiosulphate, or, preferably, sodium arsenite (Welton and Drake, *Ind. Eng. Chem.* (*Anal. Ed.*), 1929, **1,** 20), which keeps much better at the low concentrations ($N/100$) often required. For standardisation of thiosulphate solutions with potassium iodate, see Thorburn, *J. Soc. Chem. Ind.*, 1927, **46,** 355T. For the detection of very small quantities of carbon monoxide, Tausz and Jungmann (*Gas-u. Wasserfach.*, 1927, **70,** 1049; *Chem. Zentr.*, 1927 [II.], 2771) collect the iodine in a tube containing glass beads moistened with potassium iodide; one-millionth of a grm. of iodine is said to be detectable. If iodine is to be detected or estimated, there should be no rubber connections between the iodine pentoxide and the collecting vessel (Florentin and Vandenberghe, *Compt. rend.*, 1921, **172,** 391; *Analyst*, 1921, **46,** 153); glass wool and asbestos are also undesirable, as they tend to retain iodine (*loc. cit.*). When carbon dioxide is estimated, it is usually collected in standard baryta (see *Jahresber. der Chem. Tech. Reichsanstalt*, 1928, **7,** 195). An alternative way is to condense it in liquid air and measure the volume of the re-evaporated gas (Lebeau and Marmasse, *Compt. rend.*, 1924, **125,** 64).

As regards the effect of other gases, it seems that the action of hydrogen is negligibly small at 130°. Tausz and Jungmann find the maximum amount of hydrogen oxidised corresponds, in liberated iodine, with 0·008 per cent. of apparent carbon monoxide. (See also Graham and Winmill, *J. Chem. Soc.*, 1914, **33,** 1996; Davies and Hartley, *J. Soc. Chem. Ind.*, 1926, **45,** 164T); Van

daveer and Gregg (*Ind. Eng. Chem., (Anal. Ed.)*, 1929, **1**, 129; *Brit. Chem. Abstr.*, 1929, *B*, 766); Teague (*J. Ind. Eng. Chem.*, 1920, **10**, 964). Methane also is unattacked (Graham, *J. Soc. Chem. Ind.*, 1919, **38**, 10T) and likewise ethane (Vandaveer and Gregg). Teague finds that unburnt gasoline (in motor exhausts) is attacked and must be removed previously with liquid air. *n*-Pentane is attacked if present in large quantities, but only very slightly in quantities below 0·2 per cent. (Graham, *loc. cit.*). Unsaturated hydrocarbons are attacked, and should, in general, be previously removed. Levy and Pecoul, however, found that 0·01 per cent. of acetylene gave no detectable iodine. Unsaturated hydrocarbons may be removed by a variety of means. Sinnatt and Slater (*Fuel*, 1922, **1**, 241; *J. Soc. Chem. Ind.*, 1923, **42**, 133A) have shown that when bromine in potassium bromide was used (Levy, *J. Soc. Chem. Ind.*, 1911, **30**, 1437) some volatile compound passed over and reacted with the iodine pentoxide; they recommended as an alternative 10 per cent. fuming sulphuric acid. Welton and Drake recommend 60 per cent. oleum; sulphur trioxide is removed by 98 per cent. sulphuric acid, and sulphur dioxide by soda-lime. Cray and Garner (*J. Chem. Soc.*, 1924, **125**, 64) condense out heavy hydrocarbons with liquid air; Kattwinkel (*Brennst. Chem.*, 1923, **4**, 104; *Z. anal. Chem.*, 1926, **68**, 56) recommends active charcoal. Vandaveer and Gregg pass the gas through two scrubbing-towers containing chromic-sulphuric acid at room temperature, to remove unsaturated hydrocarbons, aldehydes and water. Formaldehyde is not wholly removed by chromic-sulphuric acid in the cold, but completely at 100°. The gas should in all cases be dried before passing into the iodine pentoxide.

The preparation of the iodine pentoxide is important. Tausz and Jungmann point out that in order to dehydrate iodic acid completely, it must be heated at 196°, otherwise an anhydro-acid, HIO_3O_8, is obtained (Groschuff, *Z. anorg. Chem.*, 1915, **47**, 331). With insufficiently dried pentoxide, it was found that the amounts of iodine and of carbon dioxide produced disagreed, but when it was dried at 196° the disagreement vanished. Much of the error of earlier work is ascribed to insufficient drying. Vandaveer and Gregg recommend drying at 205° to 215° for two days in a stream

of nitrogen or air, and then for two days at 150°. According to Tausz and Jungmann, it should not be packed too tightly, or traces of iodine may be liberated in the absence of carbon monoxide.

As regards the temperature for the combustion, the action apparently starts at 45° and is complete at 88° (Nowicki, from Dennis, p. 236). For practical use, temperatures from 110° to 150° have been recommended. The higher the temperature, the less chance there is of iodine solidifying out in the connecting tubes; for this reason, if no hydrogen is present, it is advisable to use a temperature of 195° (Tausz and Jungmann). The same authors recommend 120° to 130° if hydrogen is present. After the gas has been passed through, the temperature is raised to 195°, and any iodine remaining in the reaction tube and connections driven off in a current of air. The iodine pentoxide method is not suitable for gases containing large quantities of carbon monoxide, owing to the accumulation of iodine in the connections. (For other recent work, see Wood and Howarth, *Gas J.*, 1926, **175**, 787; 1926, **178**, 824; Davies and Hartley, *ibid.*, 1926, **174**, 539; 1927, **179**, 334; Schläpfer and Hofmann, *Monats. Bull. Schweiz. Ver. Gas-Wasserfachmänner*, 1927, **7**, 293, 349; *Chem. Zentr.*, 1929, [i.], 3013; *Brit. Chem. Abstr.*, 1930, *B*, 5.)

A very convenient alternative method for the fractional combustion of carbon monoxide makes use of the property which certain metallic oxides have, when suitably prepared, of catalysing the combustion of carbon monoxide at ordinary temperatures, leaving hydrogen unaffected. A successful catalyst of this type is known as "Hopcalite"; this consists of manganese dioxide, cupric oxide, cobaltic oxide and silver oxide (Lamb, Bray and Frazer, *J. Ind. Eng. Chem.*, 1920, **12**, 213). This was found to effect the combustion of carbon monoxide at as low a temperature as 0°. Subsequently a mixture was developed consisting of 60 per cent. of MnO_2 and 40 per cent. of CuO. This has been studied by Lamb, Scalione and Edgar (*J. Amer. Chem. Soc.*, 1922, **44**, 738). Provided the gas is dry, carbon monoxide is oxidised completely at room temperature, with only a trace of hydrogen being affected. With moist gas, the temperature has to be raised to

90° to 100° before oxidation of carbon monoxide is complete, and to 125° before hydrogen begins to be attacked. With concentrations of carbon monoxide of more than about 0·5 per cent., the catalyst is unsuitable, since the heat of combustion of the carbon monoxide raises the temperature sufficiently to make the hydrogen burn; under such conditions the catalyst may easily become incandescent. Although this method is not of the same precision as the iodine pentoxide method, it is very convenient, and should be of great use for the rapid estimation of small quantities of carbon monoxide in air.

Combustion of Hydrogen and Carbon Monoxide in the Presence of Methane.—In 1825, Henry (*Annals of Philosophy*, 1825, **25,** 428; Dennis, p. 192) succeeded in burning hydrogen and carbon monoxide over platinum sponge at 177° in the presence of methane. Coquillon (*Compt. rend.*, 1876, **83,** 799; 1877, **84,** 1503; 1878, **85,** 1106) introduced the use of platinum wire. A simple technique has been worked out by Whitaker (*Fuel*, 1925, **4,** 450; *J. Soc. Chem. Ind.*, 1925, **44,** *B*, 905), by which small quantities of these gases can be burnt in the presence of methane by means of platinum wire at dull-red heat. The current is turned on slowly (over a period of thirty seconds) until the appearance of the wire in the dark is "fogged-red." At this temperature, hydrogen and carbon monoxide are burnt completely in one minute, whilst methane is not attacked at all in five; methane may be subsequently burnt with the wire at yellow-white heat for two to three minutes. If the proportion of hydrogen or carbon monoxide exceeds 3 to 4 per cent., it is very difficult to maintain the wire at dull-red heat, owing to the heat produced in the combustion, and the process becomes impracticable. For very small quantities of these gases (0·25 to 0·5 per cent.) it is not necessary, in my experience, to turn on the current slowly. The simplicity of the technique recommends it; there are many possible useful applications; it might be usefully applied, for instance, in conjunction with cuprous chloride and colloidal palladium to determine such traces of carbon monoxide and hydrogen as are not removed by these reagents. I have found it of great use as a check on the completeness of oxidation of these gases by copper

oxide (*q.v.*) when very small quantities of methane are being looked for. A great advantage of this process is that it needs neither large samples of gas nor complicated apparatus. Preferential combustion of hydrogen in the presence of carbon monoxide, carbon monoxide in the presence of olefines, or olefines in the presence of paraffins is not feasible (Whitaker). Hydrogen alone can be burnt at temperatures well below red-heat (*loc. cit.*), according to Scherb (*Gas-u. Wasserfach.*, 1924, **67**, 391; *Z. anal. Chem.*, 1926, **68**, 234), at 220°. According to Ott (*Helv. Chim. Acta*, 1924, **7**, 886; *Z. anal. Chem.*, *loc. cit.*), hydrogen and carbon monoxide, alone or together, may be burnt quantitatively by platinum at 300° in the presence of methane, which can be burnt subsequently at bright red heat. The process is suitable for the quantitative determination of the three gases. The platinum, however, gets poisoned by some unascertained cause, and has to be re-activated with *aqua regia*. In view of the higher temperatures required by Whitaker's method it would appear that platinum used under ordinary conditions such as his is in this "poisoned" state. If hydrogen is burnt by platinum in the presence of ethane, a little of the latter burns as well (Scherb).

Palladium, prepared in suitable ways, removes hydrogen completely at 80° to 100°, partly by absorption and partly by oxidation. Nesmjelow (*Z. anal. Chem.*, 1909, **48**, 232; *J. Chem. Soc.*, 1909, *A.* [ii]., 519) found that appreciable methane was oxidised by palladium-asbestos at 150°; Thorburn, however (*J. Soc. Chem. Ind.*, 1927, **46**, 355T), appears to find 250° to 380° satisfactory. Grice and Payman (*Fuel*, 1924, **3**, 236) use palladium sponge heated at 100° in a water-bath, and previously heated at dull-redness in air. The palladium-asbestos is contained in glass bulbs; since these cannot be swept out with mercury, it is necessary to evacuate them before use, and subsequently to transfer the residual gas back to the measuring apparatus by means of a Topler pump or its equivalent. In this particular case, a Sprengel pump is used. Carbon monoxide inhibits the action, and must accordingly be first removed. Water, alcohol, benzene and hydrochloric acid also interfere (Hauser, *loc. cit.*, p. 1198).

Hauser (p. 1109) has extended the palladium method to the fractional combustion of carbon monoxide as well as of hydrogen. The combustion of carbon monoxide by palladium below 200° is facilitated by hydrogen, but is incomplete, formic acid and formaldehyde being produced. Hauser concluded that these are necessary intermediate products in the complete combustion, and that the presence of hydrogen or water is essential to the process. These intermediate products can, however, be completely oxidised in the cold by means of rhodium black. In the process described, the gas is passed through a U-tube, the lower portion of which is immersed in an oil-bath at 180°, and containing palladised asbestos (20 per cent.), with horizontal extremities containing rhodised asbestos (10 per cent.). The intermediate parts of the tube are filled with asbestos. Three double passages of the gas are sufficient to ensure complete oxidation. The procedure is suitable only for fairly dilute mixtures of the combustible gases with air, since large quantities of water impair the rhodised asbestos.

Perhaps the most generally satisfactory agent for the fractional combustion of hydrogen and carbon monoxide is cupric oxide at 270° to 300°, first suggested by Fresenius (*Z. anal. Chem.*, 1864, **3**, 339), established as an analytical method by Jäger (*J. für Gasbeleuchtung*, 1898, **47**, 764), and developed by Ubbelohde and de Castro (*ibid.*, 1911, **54**, 811). Terres and Mauguin (1914, **57**, 8) and others. Generally, the oxygen for the combustion of the gases is supplied by reduction of the copper oxide. If, however, oxygen is added with the gases, the copper oxide acts as a catalyst, and is not reduced (Nesmjelow, *Z. anal. Chem.*, 1909, **48**, 264; *J. Chem. Soc.*, 1909, *A*, [ii], 519). With regard to the more customary method, that is, reduction of the copper oxide, the oxidation of hydrogen appears to be complete at 220° (Ott and Scherb, *J. für. Gasbel. u. Wasservers.*, 1920, **63**, 267; *Z. anal. Chem.*, 1926, **68**, 238; also Scherb, *Gas-u. Wasserfach.*, 1924, **67**, 391; *Z. anal. Chem.*, *loc. cit.*). At 295° methane is not oxidised at all (Ott and Scherb), but, according to Terres and Mauguin, begins to be oxidised at 300°. Ethane is slightly oxidised at 295°, and very slightly at 220° (Ott and Scherb). According to Ott and Scherb, the amount of carbon dioxide obtained from the

oxidation of carbon monoxide is too small, owing to absorption of carbon dioxide. Broom, however (*J. Soc. Chem. Ind.*, 1926, **47**, 276T), appears to get correct figures for carbon monoxide when the correction for the deviation of carbon dioxide from the gas-laws is applied (*q.v.*).

Ordinary copper oxide from wire exposes an inadequate surface, making the process slow; a mixture of equal parts of short-fibre asbestos with fine copper oxide, as recommended by Donnelly, Foott and Reilly, is, in my experience, very satisfactory. A mixture of finely granulated quartz with finely powdered copper oxide is recommended by the Reichsanstalt (*Jahresber.* 1928, **7**, 195). As with the palladium method, a Topler pump or its equivalent is necessary for precise work: the long measuring tube of a Bone-Wheeler apparatus is adaptable to this function (Smith, *Gas World*, 1919, **71**, 342; *J. Soc. Chem. Ind.*, 1919, **38**, 888A; King, *Fuel*, 1922, **1**, 103; Donnelly, Foott and Reilly, *loc. cit.*). For technical work it may be unnecessary to evacuate the copper oxide tube, provided its volume is kept small. Apparatus of this type is described by Burrell and Seibert (*U.S. Bur. Mines Bull.*, 197, p. 41). Cupric oxide is regained by heating in contact with air at 500° to 600°. (For further details, see King, *loc. cit.*; Stanley and Nash, *Gas J.*, 1928, 391.)

Svedala (*Chem. News*, 1925, **130**, 1; *J. Soc. Chem. Ind.*, 1925, **44**, *B*. 116) simplifies the procedure of analysis by confining the copper oxide in a small porous crucible actually hung inside the eudiometer tube in which the gas is measured. The crucible, which is heated electrically, contains a mixture of three parts of copper oxide to one of ferric oxide. Copper oxide is regenerated by heating to redness in air. These devices may be of considerable convenience for technical work of secondary precision; good results were obtained with hydrogen, but carbon monoxide was too low, owing to absorption of carbon dioxide by the solid mass. To overcome this, a more complicated apparatus was developed, with a combustion pipette capable of being exhausted (*Chem. Listy*, 1925, **19**, 11, 73; *J. Soc. Chem. Ind.*, 1925, **44**, *B*. 342).*

* The statement in *Chem. Abstr.*, 1925, **19**, 3229, that cuprous oxide was used appears to be an error. In my experience cuprous oxide, when used in the ordinary way, is far too slow to be of any practical use.

The copper oxide method has been applied by the Reichsanstalt (*Jahresber., loc. cit.*) to the determination of small traces of carbon monoxide in air, in preference to the more usual iodine pentoxide method. Fine copper oxide at 300°, mixed with quartz as above, is used. Carbon dioxide produced is collected in standard baryta. Very small quantities of copper oxide (0·008 grm.) are sufficient.

Unsaturated hydrocarbons must be removed before the copper oxide process, as they are appreciably oxidised at 300°.

Combustion of Methane.—The combustion of methane and its homologues may be carried out, either by explosion, by slow combustion with platinum at bright yellow heat, or by copper oxide at 900° to 1,000°. At 950° the action of copper oxide is not very rapid; Wigginton (*Fuel*, 1922, **1**, 152) suggests the addition of cuprous chloride to catalyse the combustion (see Dunstan and Carr, *J. Chem. Soc.*, 1896, **12**, 48). If oxygen is added with the methane, the copper oxide is not reduced and acts merely as a catalyst; under these conditions, and with the addition of a little cuprous chloride, combustion is satisfactory at 900° (Hauser, *loc. cit.*, pp. 1193-4). Asbestos coated with a small quantity of copper oxide may be used under these conditions (Tropsch and Dittrich, *Brennst. Chem.*, 1924, **5**, 325; *J. Soc. Chem. Ind.*, 1925, **44**, *B*, 33). When the oxygen is supplied by the copper oxide, error may come in by evolution of oxygen from the latter, the equilibrium pressure of cupric oxide at 950° being 50 mm. (Bunte and Wunsch, *Gas-u. Wasserfach.*, 1923, **66**, 481; *J. Soc. Chem. Ind.*, 1924, **43**, *B*, 582). According to these authors, the oxygen may be re-absorbed by cooling down to dull-red heat and re-passing the gas slowly three times. Error due to absorption of carbon dioxide may be appreciable; according to Scherb, this absorption begins at room temperature and increases to a maximum; at a temperature corresponding to bright red heat the gas is given up again. Perquin (*Chem. Weekbl.*, 1929, **24**, 321) fills the end of the copper oxide tube with glass capillary so that the whole of the copper oxide is red-hot, and no carbon dioxide re-absorbed in the cooler parts of the tube. (For fuller details of absorption of gases by metallic oxides, see Benton, *J. Amer. Chem. Soc.*, 1923, **45**, 887.) According to Ott and Scherb, there is a possibility of

error if copper oxide comes in contact with quartz, and for this reason, as also for the protection of the quartz, it is advisable to line quartz tubes with asbestos sheet. Perquin uses a nickel tube. (See also King, Donnelly, Foott and Reilly, and Stanley and Nash.)

The platinum-wire method of effecting the combustion of methane is quicker and more convenient than the copper oxide method and, in the opinion of Dennis (*op. cit.*, p. 241), more satisfactory. Where the proportion of methane is small, it would certainly seem preferable.

Error Due to Deviation of Carbon Dioxide from the Gas-laws.—In combustions where large proportions of carbon dioxide are formed, appreciable error may arise, owing to the deviation of this gas from the gas-laws. The combustion of one volume of carbon monoxide may, for instance, give appreciably less than one volume of carbon dioxide, if the partial pressure of the latter is considerable. For details, see Burrell and Seibert (*U.S. Bur. Mines, Tech. Paper*, 54 (1913); *Bull.*, 197, pp. 85–90).

Errors Due to Solubility of Higher Paraffins.—It is to be noted that, if higher paraffins are present, a source of error is introduced on account of the solubility of these in absorbents for other gases. Ethane and propane are slightly soluble in cuprous chloride and in fuming sulphuric acid (Burrell and Seibert, *Bull.*, 197, p. 55). The higher paraffins appear to be appreciably soluble in alkaline pyrogallate (*loc. cit.*, p. 91) and in bromine water (p. 55). *n*-Butane is appreciably soluble in fuming sulphuric acid (Tropsch and Dittrich, *Brennst. Chem.*, 1925, **6**, 169; *J. Soc. Chem. Ind.*, 1926, **44**, *B*, 793). Where the proportion of higher paraffins is large, it is advisable to separate them by condensation (*q.v.*).

Heavy Hydrocarbons

Acetylene.—Acetylene has usually been absorbed either by fuming sulphuric acid, which does not distinguish it from olefines, or by ammoniacal cuprous chloride, which does not distinguish it from carbon monoxide. Lebeau, however (*Bull. Soc. Chim.*, 1924, **35**, 491), suggests the use of potassium mercuric iodide, which absorbs acetylenes without affecting carbon monoxide.

The solution is made from 25 grms. of mercuric iodide, 30 grms. of potassium iodide, and 100 c.c. of water, mixed in the absorption pipette with potassium hydroxide immediately before use. Ammoniacal silver chloride may be used for the same purpose (Hauser, p. 1178). Where acetylene is present, carbon dioxide should be absorbed by solid alkali, as acetylene is appreciably soluble in aqueous alkali (Friedrich, *Chem. Ztg.*, 1929, **53**, 706; *Brit. Chem. Abstr.*, 1929, *B*, 877).

Olefines. Olefines are usually absorbed in fuming sulphuric acid. According to Deringer (see Ott, *Helv. Chim. Acta*, 1924, **7**, 886), it is more convenient and more accurate than bromine water. A stationary pipette with glass rods is more efficient than one of the shaking type (*loc. cit.*). The addition of 0·05 per cent. of iodine to 20 per cent. oleum greatly increases its absorptive power. The reagent then, however, absorbs an appreciable amount of carbon monoxide (Thorburn, *J. Soc. Chem. Ind.*, 1927, **46**, 355T). The higher olefines are more easily absorbed by sulphuric acid than is ethylene; methods of distinguishing ethylene from its homologues on these lines have been proposed by Tropsch and von Philippovitch (*Brennst. Chem.*, 1923, **4**, 147; *J. Chem. Soc.*, 1923, *A*, ii, 509) and Dobrjanski (*Neft. Khoz.*, 1925, **9**, 565). Manning, King and Slater (*Fuel Research Tech. Paper*, 17; *Analyst*, 1928, **53**, 224) suggest treating the gas with successive lots of 2 c.c. of 87 per cent. sulphuric acid for five minutes; by this process all the higher olefines are absorbed in twenty minutes. According to Davis and Quiggle (*Ind. Eng. Chem.* (*Anal. Ed.*), 1930, **2**, 39), it is not feasible, as is sometimes stated, to separate propylenes and butylenes by this method. The absorbing power of sulphuric acid for ethylene may be increased by the addition of vanadic acid or uranyl sulphate, or preferably silver sulphate and nickel sulphate (Tropsch and Dittrich, *Brennst. Chem.*, 1925, **6**, 169; *J. Soc. Chem. Ind.*, 1925, **44**, *B*, 793). Olefines may be partially separated from one another by fractionation of their dibromides; the hydrocarbons may be regained by means of zinc-copper couple (Manning, King and Sinnatt). Olefines are completely oxidised by copper oxide at 700°. (For further details and references, see Manning, King

and Sinnatt, and Davis and Quiggle. See also "*Physical Methods.*")

Benzene and Naphthalene. With regard to benzene, Berthold (*Glückauf*, 1921, 508; from Hauser, *loc. cit.*, p. 1181) confirms Muller's method (*J. für Gasbeleuchtung*, 1898, **41**, 433) of absorption oil at − 15° to − 20°. Berl, Andress and Müller (*Z. angew. Chem.*, 1921, **34**, 125; *Analyst*, 1921, **46**, 253) recommend active charcoal at ordinary temperatures: benzene is regained by heating to 110° to 120° and distilling in steam. Bonte (*Bull. Soc. Chim. Belg.*, 1927, **36**, 485; *Brit. Chem. Abstr.*, 1927, *B*, 834) describes a process for naphthalene in which the naphthalene is frozen at 0°, dissolved in acetic acid, and the setting point of the resulting mixture determined.

Nitrogen

In nearly all the chemical processes of gas analysis, nitrogen remains inert. In practice, it is only when it is required to isolate the gases of the argon group that it is removed chemically (see Travers, *Study of Gases*, pp. 99–109). According to Leu (*Helv. Chim. Acta*, 1928, **11**, 761; *Analyst*, 1928, **53**, 763), neither calcium alone nor magnesium alone is suitable for the quantitative removal of nitrogen; the procedure recommended is to pass the gas through two steel tubes at 850° to 870°, containing magnesium turnings which have been shaken with freshly-calcined lime and mixed with 5 per cent. of finely-divided sodium. Nitrogen may be determined subsequently as ammonia by passing moist air through the tubes at 60° to 80°. For the estimation of nitrogen in residues containing nitrogen and inert gases, Trautz, Leonhardt and Scheuermann (*Z. anal. Chem.*, 1929, **78**, 341) spark with oxygen, the excess of which is subsequently absorbed; the process takes fifteen to twenty hours with a spark of 1·5 to 2 cm. in length; sparking is interrupted for five to ten minutes every hour to avoid undue heating of the vessel. Where the proportion of nitrogen is high, as, for instance, in cylinder nitrogen, this process takes inconveniently long, and in such cases, Trautz and Kipphan (*ibid.*, 350) recommend lithium at 300°. Absorption is complete

in five minutes, and an accuracy of 0·05 per cent. is claimed. Lithium was found to be preferable to calcium, potassium, or strontium. See also Blumstein (*Z. anal. Chem.*, 1930, **79**, 324) for details of a process in which magnesium is used.

Water Vapour

Hackspill and d'Huart (*Ann. Chim.*, 1926, [10], **5**, 95) have described a method for the estimation of moisture in gases whereby the water is condensed at −80° by liquid air, re-evaporated and passed over calcium hydride, which liberates hydrogen quantitatively. For the detection of very small traces of water, Heyne (*Z. angew. Chem.*, 1925, **38**, 1099) observes the effect on the surface of a mirror of alkali-metal: 0·04 per cent. of water vapour can be detected (see also under "*Physical Methods*").

AUTOMATIC GAS ANALYSIS

Automatic methods are generally limited in their scope, but may be of great use in control work. Some are based on purely physical principles; others depend on a combination of chemical and physical processes. A simple application of the hair-hygrometer to the estimation of water, formed either by the combustion of hydrogenous gases in excess of oxygen, or of oxygen in excess of hydrogenous gases, is made by Löwenstein (*Z. phys. Chem.*, 1924, **10**, 799; *J. Soc. Chem. Ind.*, 1925, **44**, *B*, 116). The apparatus is designed for continuous-flow analysis, the gas passing through a drier and then through a combustion-tube to the hygrometer, which automatically records variations in the amount of water produced. This principle should be applicable to many other processes—for instance, those involving the reaction previously mentioned between carbon monoxide and hydrogen.

An automatically-recording apparatus on a gravimetric principle is proposed by Hartung (Brit. Pat. 235,770 of 1920). The appropriate absorbent is suspended on the arm of a balance, and the gain in weight recorded.

White (*J. Amer. Chem. Soc.*, 1928, **50**, 2148) has devised an automatic apparatus based on the electrical conductivity of water with which the gas has been scrubbed. It is, of course, only applicable to gases which produce electrolytic solutions. By the addition of a combustion furnace, carbon monoxide can be brought within its scope. A process on similar lines was described by Taylor and Taylor (*J. Ind. Eng. Chem.*, 1922, **14**, 1008), who oxidised carbon monoxide by means of a catalyst and absorbed the carbon dioxide produced, in dilute ammonia. A similar principle is used by Hine (*ibid.*, 1924, **16**, 952) for small quantities of chlorine in air, although in this case the technique is not automatic. Harger (*Iron and Coal Trades Review*, 1914, **88**, 912) described a portable apparatus on a rather similar principle for the estimation of carbon monoxide.

A method has been described previously for estimating oxygen by burning with excess of hydrogen by means of a heated platinum wire. In a process developed by Larson and White (*J. Amer. Chem. Soc.*, 1922, **44**, 20; *J. Chem. Soc.*, 1922, *A*, [ii.], 591), the heat produced by this reaction is measured by a thermo-couple. The catalyst is platinised platinum at about 300°. The method is designed for "flow" analysis, and appears to be exceedingly sensitive; it is claimed that 0·001 per cent. of oxygen can be measured with an accuracy of 3 per cent. Larger proportions of oxygen can be dealt with by using a less sensitive galvanometer. If the oxygen content exceeds 0·1 per cent. some remains unburnt; this can probably be obviated by working at 400° to 500°.

In a semi-physical automatic apparatus recently brought out by the firm of Siemens and Halske (Brit. Pat. 282,080 of 1927; *Brit. Chem. Abstr.*, 1929, *B*, 197) combustible gases are burnt catalytically by means of an electrically-heated wire; the heat of the combustion raises the temperature of this wire, which acts also as an electrical thermometer, recording variations in the proportions of combustible gas. (See also Moeller, *Chem. Ztg.*, 1924, **48**, 724; Lamb and Larson, *J. Amer. Chem. Soc.*, 1919, **41**, 1908; Guasco, *Compt. rend.*, 1912, **155**, 282; Williams and Williams, U.S. Pat. 1,143,473 of 1915.)

PHYSICAL METHODS

The limitations of chemical methods of separation of gases begin to be apparent in such cases as homologous mixtures of hydrocarbons; they obviously break down completely with gases of the argon group.

Condensation Methods. The only satisfactory method for the actual separation of gases is fractional condensation and evaporation. As regards the general separation of the inert gases, little has been added to the monumental work of Ramsay and Travers (*Proc. Roy. Soc.*, 1896, **63**, 438; 1897, **64**, 183; *Phil. Trans.*, 1901, "Study of Gases," pp. 213–223). Moureu and Lepape (*J. Chim. Phys.*, 1913, 63) condensed argon, krypton and xenon by liquid air, and helium and part of the neon in coconut charcoal at the temperature of liquid air. Chlopin and Lukasuk (*Ber.*, 1925, **58**, 2392; *Z. anal. Chem.*, 1928, **73**, 155; *Chem. Abstr.*, 1926, **20**, 127) have improved this procedure and greatly simplified the apparatus; 0·0005 per cent. of helium is stated to be detectable in a sample of 200 c.c. Pénteheff (*Compt. rend.*, 1929, **189**, 322; *Analyst*, 1929, **54**, 617) determines neon in natural gas by eliminating all gases except neon and helium and determining the density of the residual mixture. The other gases are removed completely by charcoal at the temperature of liquid air; removal of these can be checked spectroscopically. The density of the helium-neon mixtures is determined by a modification of the Dumas method of weighing in bulbs (Pénteheff, *Compt. rend.*, 1927, **185**, 511; 1928, **186**, 249; 1928, **187**, 243).

The condensation method has been applied by Lebeau and Marmasse (*Compt. rend.*, 1926, **182**, 1086; *Analyst*, 1926, **51**, 366) to the estimation of small quantities of hydrogen. The gas is passed through silica-gel, previously evacuated at 150°, at the temperature of liquid air. All gases except helium, hydrogen and neon (which was not investigated) are condensed. Charcoal is not suitable as a substitute for silica-gel, owing to the hydrogen that it may retain. The same authors (*ibid.*, 1924, **125**, 64; *Analyst*, 1925, **50**, 472) estimate small quantities of carbon dioxide in air by condensation. The gas is subsequently

evaporated and measured; it can be verified by absorption in baryta. Condensation is the only satisfactory way of detecting or estimating small quantities of nitrous oxide.

Fractionation of Hydrocarbons.—A field in which the application of the condensation methods has proved of great value is the analysis of gases containing homologues of methane. These are very difficult to distinguish from one another by chemical methods; binary mixtures can be analysed, or single gases identified, by the combustion methods (p. 393), but for more complicated mixtures, chemical methods are of very little use. Moreover, the appreciable solubility of the higher paraffins in the usual liquid reagents makes it necessary to remove them prior to any precise analysis of the other gases. Burrell, Seibert and Robertson (*U.S. Bur. Mines, Tech. Paper*, 104 (1915); *Bull.*, 197, p. 96) describe a condensation technique for natural gas. The chief complication is the behaviour of methane: in the presence of large quantities of condensible gas it is partially condensed, and is not wholly removed on subsequent pumping. In order to remove it completely, the gas has to be re-evaporated and recondensed one or more times. Methane can be removed much more quickly if the liquid air has been allowed to stand for some time so that its boiling point has risen to approach that of oxygen. A manometer in the system is valuable, as, during the pumping off process, it remains steady while a gas is being evaporated: a sudden drop in pressure indicates that the whole of the gas in question has been evaporated.

Fractionating columns for liquefied gas have been described by Lucas and Dillon (*J. Amer. Chem. Soc.*, 1928, **50**, 1460) and by Frey and Yant (*Ind. Eng. Chem.*, 1927, **19**, 489; *Analyst*, 1927, **51**, 359). Davies (*Amer. Petroleum Inst., Bull.*, 9 (1928), p. 11; *J. Amer. Chem. Soc.*, 1928, **64**, 2779) has introduced a silvered jacket; the fractionating column consists of a narrow glass spiral surrounded by a silvered vacuum jacket. Heat is supplied electrically by a coil immersed in the liquefied gas. The process is said to be almost as easy as the fractionation of ordinary liquids (Davies, *Ind. Eng. Chem.*, (*Anal. Ed.*), 1929, **1**, 61). This paper gives a complete chart of the boiling points of hydrocarbons

between −162° and +13°. (See also Lebeau and Damiens, *Compt. rend.*, 1913, **156**, 144, 325, 797; Shepherd and Porter, *J. Ind. Eng. Chem.*, 1923, **15**, 1143; *Analyst*, 1924, **49**, 50; Tropsch and Dittrich, *Brennst. Chem.*, 1925, **6**, 169; Coffin and Maass, *J. Amer. Chem. Soc.*, 1928, **50**, 1427; Perquin, *Chem. Weekbl.*, 1929, **24**, 321).

Condensation Method for Very Small Quantities.—A condensation method on a different principle, for the analysis of very small samples, has been worked out by the Research Staff of the General Electric Co. (*Proc. Phys. Soc.*, 1921, **33**, 287; *J. Chem. Soc.*, 1921, *A*, [ii.], 591). The gases are condensed, and the temperature slowly raised, the pressure being continuously measured. Each constituent gas can be recognised with certainty by the form of its characteristic curve connecting vapour pressure and temperature below boiling point. The amount of each constituent is proportional to the pressure it exerts after evaporation. The pressure ranges from 0·001 mm. to 0·1 mm.; 0·0005 mm. of carbon dioxide, for example, is detectable. The method is applicable, properly, only to vapours; permanent gases can, however, be detected to the extent of 0·05 mm. In some cases, permanent gases can conveniently be converted into vapours; hydrogen and carbon monoxide, for instance, can be converted into water and carbon dioxide by means of a red-hot copper wire coated with cuprous oxide. Oxygen is removed in the same process. Pressures are measured on a Pirani gauge, as modified by Hale (*Amer. Electrochem. Soc. Trans.*, 1911, **20**, 243). This instrument is based on the cooling of a platinum wire carrying a current; as the pressure of the gas decreases, its thermal conductivity also decreases, and the temperature and, consequently, the resistance of the wire increase. The instrument is sensitive to 0·0001 mm. pressure. Other condensation methods for small quantities of gas have been described by Langmuir (*J. Amer. Chem. Soc.*, 1912, **34**, 1310); Ryder (*ibid.*, 1918, **40**, 1657) and Hamburger (*Z. anal. Chem.*, 1918, **57**, 121; *J. Soc. Chem. Ind.*, 1918, **37**, A, 446.).

Density Methods. After boiling point, the physical property which is the most applicable to analytical purposes is density.

Density methods are the more delicate in proportion as the densities of the constituents of the mixture differ. A number of types of density-balance have been designed (Steele and Grant, *Proc. Roy. Soc.*, 1909, *A*, **82**, 580; Gray and Ramsay, *ibid.*, 1910, *A*, **84**, 536; Aston, *ibid.*, 1913, *A*, **89**, 439; Taylor, *Phys. Rev.*, 1917, **10**, 653). These instruments depend on the buoyancy of a hollow bulb balanced in an atmosphere of the gas under measurement. In a simple form devised by Masson and used in some high-precision work (Masson and Dolley, *Proc. Roy. Soc.*, 1923, *A*, **106**, 524), a beam, to one end of which is sealed a glass bulb of 0·2 cm. diameter and to the other a counterpoise lump of glass, is balanced on a horizontally-stretched silica fibre. This beam swings inside a glass tube which contains the gas and is immersed in a water-bath of known temperature. The pressure of the gas is adjusted until a mark on the bulb viewed through a telemeroscope is brought on some arbitrary line. The gas is compared with some other gas of known density, usually oxygen; the density-ratio of the gases is the reciprocal of the ratio of the pressures measured.* The apparatus is accurate to about 1 part in 5,000. For cruder control work in industry, automatically-indicating gas-balances have been in use for many years. Stock (*Z. Phys. Chem.*, 1928, **139**, 47; *Brit. Chem. Abstr.*, 1928, *A*, 417) describes a density balance in which the beam contains a piece of soft iron and can be controlled by an electromagnet. An ammeter connected with the windings of the magnet is calibrated to read directly in terms of density.

For a method of measuring density based on the rate of effusion through a small orifice, see Biluchowski and Kling (*Metan.*, 1917, **1**, 13; *Chem. Zentr.*, 1919, [iv.], 709).

Velocity of Sound.—The velocity of sound in a gas depends on its density. A primitive apparatus on this principle for the analysis of mine air was made in 1884 by Forbes and Blaikley (see discussion on Griffiths' apparatus below). The instrument consisted of an organ pipe whose pitch varied with the density of the gas within it. It could, of course, be used only in cases where very large samples were available, as in the case of air. Geberth (*J. Ind. Eng. Chem.*, 1923, **15**, 1277; *Analyst*, 1924, **49**, 109) has

* That is, if the two gases deviate equally from Boyle's Law.

described a small and simple apparatus on a similar principle, which is capable of indicating hydrogen with an accuracy of 0·1 per cent. The gas is contained in a closed cylinder whose length is adjusted until resonance is obtained with an electrically-maintained vibrating diaphragm. Griffiths (*Proc. Phys. Soc.*, 1927, **39**, 300) has designed a precision apparatus on the same general lines, employing supersonic vibrations.

Thermal Conductivity.– One of the most useful advances in recent years is the development of the method of thermal conductivity. The thermal conductivity of a gas, which increases with decreasing density, is conveniently measured by means of a wire surrounded by the gas and carrying a current. The thermal conductivity of the gas determines the rate at which the wire loses heat, and accordingly its temperature and electrical resistance (see Schleiermacher, *Wied. Ann.*, 1888, **34**, 623 ; 1889, **36**, 346 ; Goldschmidt, *Phys. Z.*, 1911, **12**, 417 ; Eucken, *ibid.*, 1101). The principle was applied to gas analysis by Sowzee in 1880, developed by Koepsel (*Ber. Phys. Ges.*, 1908, **10**, 814 ; 1909, **11**, 237 ; *Z. Chem. App.*, 1908, **3**, 377 ; *Chem. Abstr.*, 1908, **2**, 3212 ; 1909, **3**, 1108, 2646) and by Shakespear (see Daynes, *Proc. Roy. Soc.*, 1920, *A*, **97**, 273). A useful summary of the method is given by Weaver and Palmer and others (*J. Ind. Eng. Chem.*, 1920, **12**, 359, 894).

The wire which is surrounded by the gas is usually made one arm of a Wheatstone bridge. The method can be made very sensitive, but is dependent on calibration against gases of known composition. It is, in general, suitable for binary mixtures only ; in some cases, however, since heavy gases exert a proportionately greater influence than the lighter ones, ternary mixtures can be dealt with. According to Weaver and Palmer (*loc. cit.*), the estimation of carbon dioxide in mixtures with hydrogen and nitrogen is almost independent of the ratio of the two last gases. The qualitative composition of the gas must, in general, be known. The most important applications of the conductivity method would seem to be for control analyses in technical processes ; the instruments are usually self-indicating, and can be made to record. One recent technical application is the estimation of

sulphur dioxide in flue-gas (Gruss, *Z. angew. Chem.*, 1925, **38**, 488; *Z. anal. Chem.*, 1926, **68**, 53). The scope of the method may be extended by the addition of chemical processes; methane in air, for instance, can be determined much more accurately if the gas is first passed through a copper oxide furnace, to convert the methane into the heavier carbon dioxide. In the laboratory the conductivity method should be of use in the cases where chemical analysis is unsatisfactory, such as the estimation of nitrous oxide in the presence of combustible gases and oxygen, or mixtures of homologous hydrocarbons. It is also particularly applicable to mixtures of the inert gases.

Refractivity. This is another physical property applicable to gas analysis. For any precision in the analysis, a highly sensitive method of measuring refractivity is demanded. A suitable instrument for the purpose is the Rayleigh interferometer; this has been used with success by the U.S. Bureau of Mines (Seibert and Harpster, *Tech. Paper*, 185 (1913); *Bull.*, 197, p. 74). A laboratory instrument was accurate to 0·02 to 0·03 per cent., and a portable one to 0·2 to 0·3 per cent., the gas to be estimated being carbon monoxide or methane in air. (See also Haber, *Z. angew. Chem.*, 1906, **19**, 1418; Mohr, *ibid.*, 1912, **25**, 1313; Löwe, *Phys. Z.*, 1910, **11**, 1047; Kuppers, *Glückauf*, 1913, **49**, 47.)

Spectroscopy. The emission-spectrum of a gas is a precise and delicate test for minute quantities, and is of particular value in connection with the inert gases, constituting one of very few positive tests for gases with no chemical reactions to identify them (see Travers, *op. cit.*, chap. XXII.; Hempel, *Gasanalytische Methoden*, p. 151, *et seq.*). Moureu and Lepape in 1911 introduced a spectrophotometric method for the quantitative estimation of krypton and xenon, and improved and simplified it in 1922 (*Compt. rend.*, 1911, **152**, 691; 1911, **153**, 740; 1922, **174**, 908; *J. Chem. Soc.*, 1911, *A*, [ii.], 439, 1134; 1922, *A*, [ii.], 394). The principle is the comparison of the intensity of certain lines in the krypton and xenon spectra with neighbouring lines in the argon spectrum. For the detection of hydrogen in nitrogen or inert gases, spectroscopic observation can detect 0·005 per cent. at a pressure of 0·05 to 0·06 mm.; the hydrogen red line is particu-

larly conspicuous (Heyne, *Z. angew. Chem.*, 1925, **38**, 1099; *Z. anal. Chem.*, 1928, **73**, 153). For a recent method based on high dispersion infra-red spectroscopy, used in connection with the methyl halides, see Bennett and Meyer (*Phys. Rev.*, 1928, **32**, 888).

Electrical Methods.—Small traces of electronegative gases (oxygen or water-vapour) have a marked effect on the point-discharge in nitrogen or inert gas (Pirani, *Wissenschaftliche Veröffentlichungen aus dem Siemens-Konzern*, 1920, **1**, 167). According to Heyne (*loc. cit.*), quantities as small as 10^{-4} per cent. can be detected by this means. Electropositive gases have a much smaller effect, and can only be detected in quantities of the order of 0·1 per cent.

Another very sensitive test for impurities in nitrogen consists in observing the after-glow after the electric discharge. Quite pure nitrogen gives no after-glow, but the presence of some impurities in quantities of $1{\cdot}5 \times 10^{-5}$ per cent. can cause it to appear. 10^{-3} per cent. of oxygen, water, or carbon dioxide give a very strong after-glow. Argon and hydrogen have no effect (Heyne).

The electrical discharge has been applied also to the actual purification of gases. Loebe and Ledig (*Z. tech. Phys.*, 1925, **6**, 287; *Z. anal. Chem.*, 1928, **73**, 155; *Chem. Zentr.*, 1925, [ii.], 2009) remove oxygen, water and carbon dioxide by means of the luminous discharge between electrodes of an alkali metal. The amount of such gases may be estimated by measuring the pressure before and after the process.

A purely physical electrical method of separating gases has been worked out by Skaupy and Bobek in connection with the inert gases (*Z. tech. Phys.*, 1925, **6**, 284; *Z. anal. Chem.*, 1928, **73**, 155; *Chem. Zentr.*, 1925 [ii.], 2009). Under the continuous discharge, the less ionisable of two gases accumulates at the anode; samples round the anode can be removed by a Töpler pump. The efficiency of the process is greatest when the pressure of gas is lowest, and when the discharge is strongest. By this method, it is claimed, samples of helium containing 8 per cent. and 12 per cent. of argon were purified so that the argon content was reduced to 0·25 per cent. or less.

SUMMARY OF THE PROGRESS OF GAS ANALYSIS

Perhaps the most marked feature of the developments in gas analysis in the last ten years is the number of specialised physical and semi-physical techniques that have been brought out. These have, in general, the advantages of economy in time and in skilled manipulation, and it seems probable that much of the future development in gas analysis is to be looked for along these lines. On the other hand, such methods are usually applicable only to simple mixtures of known qualitative composition, and usually require calibration against the absolute chemical-volumetric methods.

With regard to the more regular methods, the most important chemical advances are in the various fractional combustion methods which, although not new in principle, have only recently come into widespread use. The problem of the precise determination of hydrogen, carbon monoxide and methane is certainly solved by these methods, although there is still scope for simplifying the procedure and apparatus necessary. With regard to higher hydrocarbons, on the other hand, it seems that the chemical method is unlikely to provide a solution to the problem, which is, however, being found along purely physical lines (p. 399).

There is still much scope for perfection of many of the commonest analytical processes. A thoroughly satisfactory absorbent for oxygen is yet to be discovered. An absorbent for methane would be of great use, as would also an absorbent or a specific test for nitrous oxide. The likelihood of these last being found does not appear great, but research might be profitably directed to the investigation of possible oxygen absorbents.

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